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Long Island Sound Study
Final Grant Report

EPA Assistance Award No: LI-972309090
Project Title: Research to Fulfill the Long Island Sound Study's Goals and Targets (NYSG Share)
Project Period: 10/01/2009 – 09/30/2013, extended to 3/30/2014

1. Project Summary: *(A concise statement of the goals and objectives, methods employed, essential outcomes and results, and conclusions of the study – not to exceed two pages. The summary must be understandable without reference to the body of the report.)*

This project was a joint effort of the New York and Connecticut Sea Grant programs. It had two tasks, the first of which was to administer a competitive research program for the Long Island Sound Study. The process used input from the LIS Science and Technical Advisory Committee (STAC) to define the high priority topics for a Request for Proposals. The RFP was broadly distributed and resulted in 40 pre-proposals. The submissions were reviewed by a Panel and a subset of the most promising pre-proposals were invited as full proposals. Twelve full proposals were accepted and sent for mail peer review to appropriate academic experts. A Panel considered the proposals and their peer reviews, and five high priority and high quality submissions were selected for funding. A sixth highly-rated project was partially supported as a pilot effort. Each Sea Grant program served as the primary administrator for three of the projects. A press release announced the results of the competition and various articles and presentations about the funded projects kept the LISS committees and/or the public informed about their progress and results. The topics of the projects under this award included: the role of gelatinous zooplankton, toxic dinoflagellate blooms, nitrogen sources and fates in embayments, nitrogen removal Best Management Practices, effects of eutrophication on habitats, and nitrogen removal capacity of estuaries. The research results were interesting and valuable, with several journal publications, graduate student theses, and many presentations sharing the findings. The purpose of the research, overall, was to help achieve the vision and the goals of the LISS CCMP and subsequent policy agreements by providing needed scientific information. The second task of this award was to financially support the meetings of the STAC. This group met 2-3 times a year, alternating between NY and CT locations. The STAC advises the Management Committee on science and technical aspects of LISS's goals

2. Grantee Organization and Contact Name:

The Research Foundation of SUNY on behalf of New York Sea Grant Institute
Ms. JeanAnn Johnston, Fiscal Officer, New York Sea Grant

3. Project Description: *(Provide a brief overview of the project, including a reiteration of the goals and objectives of your project and the management implications of your work.)*

Task 1: In the conduct of this project, the Connecticut and New York Sea Grant programs (CTSG and NYSG) jointly administered a competitive research program to address the needs of the Long Island Sound Study (LISS). The *Long Island Sound Comprehensive Conservation and Management Plan* (CCMP) and the *Research, Monitoring, and Assessment Needs to Attain LISS Goals and Targets* (aka Needs Assessment) report, served as the foundation for the program, with further input from the LISS Science and Technical Advisory Committee (STAC) on identifying the highest research priorities.

The objective of this project was to identify and fund high priority, high quality research needed in order to best achieve the vision and the goals of the LISS CCMP and subsequent policy agreements.

A very similar effort was previously underway with EPA LISS funds from 2008 (Grant LI-972417080 to NYSG). Feedback from researchers and the STAC about the processes and outcomes of that project were used to fine-tune this one.

Task 2: This award also included funds to support meetings of the Long Island Sound Science and Technical Committee (LIS STAC).

4. Activities and Accomplishments: *(Report on project activities over the entire period of funding, describing in detail the achievements and outcomes with respect to the stated project purposes and objectives.)*

Work Plan Activity	Deliverable(s)	Timeline	Expected Output	Actual Output	Expected Outcome	Actual Outcome
Task 1: Research						
A. Select research projects through an open, competitive,	1. Develop priority list of topics for the Call for Pre-proposals with the STAC. 2. Distribute Call for Pre-proposals.	1. February 2010 STAC meeting. 2. 5/7/2010	1-2. Deliverable: Call for Pre-proposals.	1-2. The Call for Pre-proposals was developed and released on 5/7/2010, as scheduled.	Highest priority research and assessment work relevant to the LISS CCMP and	Six high quality, high priority projects were selected and funded (see list below this table). Their results are useful and relevant to the LISS. Several publications have already

peer reviewed process.	3. Pre-proposals due.	3. 6/25/2010	3-7. Deliverable: List of research selected for funding.	3-7. Forty pre-proposals were received by the 6/25/2010 due date. A Pre-proposal Review Panel was convened, resulting in the invitation of 14 full proposals. Notifications to all authors took place on 8/6/2010 as planned. Panel Summaries and Full Proposal Instructions were developed and distributed. Twelve proposals were received by the 10/1/2010 deadline. Three mail peer reviews were secured for all but one proposal (which had 2). The Review Panel met by video conference on 12/13/2010. Five proposals were selected for funding, and PIs were notified in early January 2011, a bit later than targeted but not problematic. The PIs of all the submissions were provided with their masked peer reviews and a Panel Summary. A sixth effort was supported, after negotiation, as a pilot project with reduced funding. Throughout the review process, potential conflict of interest was minimized and managed. A news release about the projects was developed and yielded a newspaper article and several web postings	policy agreements is defined, openly-solicited, and selected for funding using a well-developed, respected process that is fair and technically-based.	appeared in peer reviewed journals. Graduate student theses have been produced, and many presentations shared the research results. The final reports from 5 of the projects are attached. The pre-final report from the Anisfeld project is also attached, since that final report has not yet been submitted. It is expected in summer 2014.
	4. Review panel screens pre-proposals, full proposals invited.	4. 8/2/2010				
	5. Full proposals due.	5. 10/1/2010				
	6. Mail peer review interval.	6. 10/12/2010-11/9/2010				
	7. Review panel meets, funding decisions made, PIs notified.	7. 11/22/2010-12/8/2010				
B. Administer the selected projects	1. Award grant funds for Year 1.	1. 3/1/2011	1-7. Deliverables: 2 progress reports and 1 final report per research project.	The start date for the three projects administered by NYSG was 3/1/2011. Progress reports for those projects were requested and received to coincide with NYSG's reporting to EPA. The Gobler-led project reached completion on 2/28/2013, as originally scheduled. Lonsdale's and Hanson's projects were extended to 10/31/2013 and 12/31/2013, respectively. NYSG	New science-based information was developed that will inform decision-making and actions towards	

	2. 10-month progress report requested and due.	2. 1/15/2012		contributed $11,391 of its federal NOAA award by continuing to support some of the graduate students involved in the NYSG-administered research projects after their EPA funds were depleted. The 3 projects administered by CTSG were given their awards between 3/1/2011 and 5/1/2011. The pilot project (led by Tobias), however, did not start its field work until summer 2012. That project ended on 2/28/2013. The projects led by Anisfeld and Vaudrey were both extended to 2/28/2014.	reaching the vision and goals for Long Island Sound.	
	3. Evaluate for sufficient progress.	3. 2/15/2012				
	4. Award grant funds for Year 2.	4. 3/1/2012				
	5. 22-month progress report requested and due.	5. 1/15/2013				
	6. Research project end date.	6. 2/28/2013				
	7. Final report requested and due	7. 4/1/2013				

This is the list of research projects selected and funded by this effort (see Table above). The first three (Lonsdale, Gobler, and Hanson) were managed by NYSG.
R/CE-31-NYCT ***"The influence of gelatinous zooplankton on nutrient cycles, hypoxia, and food webs across Long Island Sound"***
PIs: Darcy Lonsdale and Christopher Gobler. Start Date: 3/1/2011 End Date: 10/31/2013

R/CMB-38-NYCT ***"Phase shifts among primary producers within Long Island Sound: Will anthropogenic stressors continue to expand the niche of PSP- and DSP-producing dinoflagellate blooms?"*** PI: Christopher Gobler. Start Date: 3/1/2011 End Date: 2/28/2013

R/CTP-44-NYCT ***"Sources and fate of nitrogen in the North Shore embayments"*** PIs: Gilbert Hanson and Teng-Fong Wong. Start Date: 3/1/2011 End Date: 12/31/2013

R/CTP-45-CTNY ***"Systematic evaluation of nitrogen removal by BMPs in the Long Island Sound watershed"*** PIs: Shimon Anisfeld and Gaboury Benoit. Start Date: 4/1/2011 End date: 2/28/2014

R/CE-32-CTNY ***"Comparative analysis of eutrophic condition and habitat status in Connecticut and New York embayments of Long Island Sound***" PIs: Jamie Vaudrey and Charles Yarish. Start Date: 3/1/2011 End Date: 2/28/2014

R/CTP-46-CTNY (Pilot project) ***"Nitrogen removal capacity of Connecticut estuaries: Assessing distribution and controls"*** PI: Craig Tobias. Start Date: 5/1/2011 End Date: 2/28/2013

Work Plan Activity	Deliverable(s)	Timeline	Expected Output	Actual Output	Expected Outcome	Actual Outcome
Task 2: STAC						
Provide financial support for STAC meetings	Work with STAC co-chairs to handle invoices associated with costs of the STAC meetings. Checks distributed to appropriate recipients.	Within several weeks of STAC meetings.	Meetings held as planned, with successful functioning of the STAC. Payments made to STAC participants and vendors, for appropriate and documented costs.	NYSG paid expenses related to the following STAC meetings: 11/19/2010 (NY) 2/11/2011 (CT) 7/8/2011 (NY) 11/18/2011 (CT) 2/10/2012 (NY) 6/14/2012 (CT) 11/16/2012 (NY)	Increased scientific validity of, and progress towards, fulfulling the LIS CMP.	The LIS Office and the LIS Management Committee benefitted from the scientific input from the STAC on several important issues related to the LIS CMP.

5. Modeling:
N/A, this award was not specifically a modeling effort or exercise.

6. Summary of Findings:
See the attached abstracts for the research projects.

7. Conclusions:
It is important for the LISS to support research-based projects that fill information gaps. Utilizing the NY and CT Sea Grant programs, who have great experience in the processes of soliciting, reviewing, selecting, and managing competitively-funded research projects, is a good mechanism to accomplish this.

8. Presentations/Publications/Outreach:
(Describe any major presentations you have made about your project and discuss any outreach efforts related to this project. If applicable, report any articles or papers resulting from this project appearing in scientific, technical, or professional journals. Copies of publications and reprints that have not previously been submitted to the LISS should be enclosed with the report. The grantee is encouraged to continue to notify the Project Officer of any papers that are published based on the research conducted under this agreement.)

Publications:

Hattenrath-Lehmann, T.K. and C.J. Gobler (2011) Allelopathic inhibition of competing phytoplankton by North American strains of the toxic dinoflagellate, *Alexandrium fundyense*: evidence from field experiments, laboratory experiments, and bloom events. *Harmful Algae* 11:106-116.

Hattenrath-Lehmann, T.K., M.A. Marcoval, D.L. Berry, S. Fire, Z. Wang, S.L. Morton, and C.J. Gobler (2013) The emergence of *Dinophysis acuminata* blooms and DSP toxins in shellfish in New York water. *Harmful Algae* 26:33-44.

Hattenrath-Lehmann, T.K., M.A. Marcoval, D.L. Berry, S.L. Morton, and C.J. Gobler. A tale of two dinoflagellates: Co-occurring blooms of the DSP- and PSP-producing dinoflagellates, *Dinophysis acuminata* and *Alexandrium fundyense*, in a New York estuary and toxicity of shellfish. (in prep.)

Hattenrath-Lehmann, T.K., R.B. Wallace, F. Koch, H. Mittelsdorf, J.A. Goleski, J.L. Smith, D.A. Anderson, and C.J. Gobler. The effects of elevated CO_2 on the growth and toxicity of field populations and cultures of the PSP-producing dinoflagellate, *Alexandrium fundyense*. *Limnology and Oceanography* (in prep.)

Sílvia Anglès, E.G., T.K. Hattenrath-Lehmann, and C.J. Gobler (2012) *In situ* life-cycle stages of *Alexandrium fundyense* complex during a bloom development in New York (USA). *Harmful Algae*, 16:20–26.

Treible, L.M., D.J. Lonsdale, and C.J. Gobler. The role of ctenophores in nutrient regeneration in Long Island Sound. *Mar. Ecol. Prog. Ser.* (in revision)

Vaudrey, J.M.P. (2012) The Breathing of the Bays. *Wrack Lines* (Spring/Summer 2012): 5-7.

Vaudrey, J. and C. Yarish. (2012) Taking the Pulse of Long Island Sound's Embayments. Short article for the Long Island Sound Study's *2012 Sound Health Indicators Report.*

Vaudrey, J.M.P., A. Chlus, A. Branco, C. Yarish, and J. Kremer. Nitrogen inputs to Long Island Sound embayments from the NLM (Nitrogen Loading Model): estimates vary with methods used for estimating population. (in prep.)

Presentations:

Durand, J.M, C.R. Young, G.N. Hanson, and T.F. Wong (2012) Submarine groundwater discharge (SGD) in Stony Brook Harbor, NY. American Geophysical Union, San Francisco, CA; December 7 (poster).

Gobler C.J., and T.K. Hattenrath-Lehmann (2013) Continued expansion of *Alexandrium* blooms and PSP across Long Island Sound. Long Island Sound Research Symposium. Port Jefferson, NY; April.

Gobler, C.J., T.K. Hattenrath-Lehmann, and S.L. Morton (2012) The distribution, causes, and impacts of *Alexandrium fundyense* blooms in coves, near shore, and open water regions of Long Island Sound. Long Island Sound Study Science and Technology Advisory Committee meeting, Stony Brook, NY; February 2.

Gobler, C.J., T.K. Hattenrath-Lehmann, Y.Z. Tang, and F. Koch (2012) Tragedy of the commons: Eutrophication, acidification, and the expansion of HABs across Long Island, NY, USA. 15th International Conference on Harmful Algae, Changwon, Korea; October.

Hattenrath-Lehmann T.K., and C.J. Gobler (2011) Factors promoting blooms of the PSP- and DSP-producing dinoflagellates, *Alexandrium fundyense* and *Dinophysis acuminata*, in Long Island Sound. New England Estuarine Research Society symposium, Port Jefferson, NY; May.

Hattenrath-Lehmann, T.K., and C.J. Gobler (2012) The PSP- and DSP-producing dinoflagellates, *Alexandrium fundyense* and *Dinophysis acuminata*, and shellfish toxicity in New York estuaries. Stony Brook Southampton Coastal & Estuarine Research Program Environmental Symposium. Southampton, NY; April (poster).

Hattenrath-Lehmann, T.K., J.A. Goleski, H. Mittelsdorf, and C.J. Gobler (2013) The expansion of the PSP- and DSP-producing dinoflagellates, *Alexandrium fundyense* and *Dinophysis acuminata*, and shellfish toxicity across Long Island. Stony Brook Southampton Coastal & Estuarine Research Program Environmental Symposium. Southampton, NY; April (poster).

Hattenrath-Lehmann, T.K., S.L. Morton, and C.J. Gobler (2011) A tale of two dinoflagellates: Co-occurring blooms of the PSP- and DSP-producing dinoflagellates, *Alexandrium fundyense* and *Dinophysis acuminata*, in a New York estuary. 6th Symposium on Harmful Algae in the U.S, Austin, TX; November.

Hattenrath-Lehmann, T., S.L. Morton, and C.J. Gobler (2012) The emergence of toxic *Dinophysis acuminata* blooms in a New York estuary. 15th International Conference on Harmful Algae, Changwon, Korea; October (poster; Maureen Keller Award, Best Student Poster).

Lucena, Z. (2014) Factors controlling biogeochemical removal of nitrogen in constructed wetlands. Conference on green infrastructure and water management in growing metropolitan areas, Tamp, FL: January 14.

Lucena, Z. (2014) Factors controlling biogeochemical removal of nitrogen in constructed wetlands. Yale University's Hixon Center for Urban Ecology, fellow presentation, New Haven CT; February 19.

Lucena, Z. (2014) Factors controlling biogeochemical removal of nitrogen in constructed wetlands. Connecticut Association of Wetland Scientists: 2014 Annual Meeting, Southbury, CT; March 20.

Tobias, C., P. Plummer, C. Cooper, D. Cady, and V. Rollinson (2013) Nitrogen removal capacity of Connecticut estuaries. Long Island Sound Research Conference, Port Jefferson, NY; April 19.

Treible, L.M., D.J. Lonsdale, and C.J. Gobler (2012) The role of ctenophores in nutrient regeneration in Long Island Sound, NY. "Long Island Marine Habitats", Stony Brook University, Southampton, NY; November 7.

Treible, L.M., D.J. Lonsdale, and C.J. Gobler (2013) The role of ctenophores in nutrient regeneration in Long Island Sound, NY. ASLO Aquatic Sciences Meeting, New Orleans, LA; February 13.

Treible, L.M., D.J. Lonsdale, and C.J. Gobler (2013) The role of ctenophores in nutrient regeneration in Long Island Sound, NY. Long Island Sound Research Conference, Port Jefferson, NY; April 19.

Vaudrey, J.M.P. (2013) Using nitrogen budgets as a tool to more effectively manage Long Island Sound embayments. 2nd Workshop on Using Cultivated Seaweed and Shellfish for Nutrient Bioextraction in LIS and the Bronx River Estuary, Mamaroneck, NY.

Vaudrey, J.M.P. (2013) Marine ecosystem ecology. "Women In Science" program for middle school girls, The Sound School.

Vaudrey, J.M.P. (2014) Eutrophic condition and habitat status in Connecticut and New York embayments of Long Island Sound. Southern Connecticut State University.

Vaudrey, J.M.P. (2014) The breathing of the bays: A journey into green water. Faulkners Light Brigade Lecture Series, Guilford.

Vaudrey, J.M.P. and C. Yarish (2011) Comparative analysis of eutrophic condition and habitat status in Connecticut and New York embayments of Long Island Sound. Presentation to the LISS STAC; November 18.

Vaudrey, J.M.P. and C. Yarish (2012) Comparative analysis of eutrophic condition and habitat status in Connecticut and New York embayments of Long Island Sound. Presentation to the LISS STAC; November 16.

Vaudrey, J.M.P. and C. Yarish (2013) Comparative analysis of eutrophic condition and habitat status in Connecticut and New York embayments of Long Island Sound. New England Estuarine Research Society Spring Meeting; April 11-13.

Vaudrey, J.M.P. and C. Yarish (2013) Nitrogen loading to embayments of Long Island Sound: method review and potential utility to management. Presentation to the Long Island Funders Collaborative Meeting, New York City, NY; March 1.

Weber, L. (2012) Reducing hypoxia levels in Long Island Sound with Connecticut constructed wetlands. American Museum of Natural History's Student Conference on Conservation Science, New York, NY, October 10-13.

Weber, L. (2013) Examining the efficacy of Connecticut constructed wetlands as a stormwater Best Management Practice. Connecticut Association of Wetland Scientists: 2013 Annual Meeting, Southbury, CT; March 21.

Weber, L. (2013) Inter-storm variability in nitrogen removal in a Connecticut constructed wetland. Yale University's Hixon Center for Urban Ecology Fellow Presentation, New Haven, CT; March 4.

Weber, L. (2013) Inter-storm variability in nitrogen removal in a Connecticut constructed wetland. Yale University's Master of Environmental Science Colloquium, New Haven, CT; April 19.

Yarish, C., and J. Vaudrey (2011) Comparative Analysis of Eutrophic Condition and Habitat Status in Connecticut and New York Embayments of Long Island Sound. LISS STAC meeting, West Haven, CT; November 18.

Young, C. (2011) Fate of nitrogen in subterranean estuaries of Long Island Sound embayments. Gordon Research Conference, Chemical Oceanography Section, Andover, NH; August 17 (poster).

Young, C., and G. Hanson (2011) Subterranean estuary nitrogen transport into Long Island Sound embayment. American Geophysical Union Fall Meeting 2011, San Francisco, CA; December 9 (poster).

Young, C., and G. Hanson (2012) Fate of nitrogen during oxic submarine groundwater discharge into Stony Brook Harbor, New York. Goldschmidt Geochemistry Conference, Montreal, Canada; June 26 (poster).

Young, C.R., and G.N. Hanson (2011) Groundwater nitrate attenuation during transport through a subterranean estuary in a Long Island Sound embayment. American Geophysical Union, San Francisco, CA; December 5-9 (poster).

Young, C.R., and G.N. Hanson (2012) Fate of nitrogen during oxic submarine groundwater discharge into Stony Brook Harbor, New York. International Association of Hydrogeologists, Niagara Falls, Canada; September 21.

Young, C.R, and G.N Hanson (2014) N_2O formation mechanisms in sandy unconfined coastal aquifers. Goldschmidt Geochemistry Conference, Sacramento CA; June 8-13.

Young, C.R, J. Durand, and G.N. Hanson (2012) Nitrogen transformations during oxic SGD in Stony Brook Harbor, NY American Geophysical Union, San Francisco, CA; December 3-7 (poster).

Young, C.R., J.M. Durand, A. Rajendra, and G.N. Hanson (2012) Nutrient dynamics in a subterranean estuary over two spring neap tidal cycles. American Geophysical Union, San Francisco, CA; December 7 (poster).

Young, C.R, J. Tamborski, A.D. Rogers, and G.N Hanson (2013) Distribution of submarine groundwater discharge into Port Jefferson Harbor, Long Island Sound, NY. American Society of Limnology and Oceanography, New Orleans, LA; February 18-22.

Other Outreach:
A press release about the projects to be funded was distributed on 3/8/2011. *The Day*, a CT newspaper, ran a story and numerous online publications picked up the posting from EurekAlert.

In 2012, for the Center for Talented Youth (CTY) Science and Technology Series, graduate student Laura Treible led students in plankton tows and showed them various species under the microscope. On a “VIP” cruise on the R/V Seawolf, Treible, Darcy Lonsdale, and David Rawitz performed plankton tows and showed the Stony Brook University President, members of the President’s Council, and the News 12 Network owner the organisms under a microscope.

12. Other Information:
There were several graduate students funded as part of the NYSG-administered projects. Two thesis are already complete, and they are:

Treible, Laura (2013) The Role of the Ctenophore *Mnemiopsis leidyi* in Nutrient Cycling in Long Island Sound, NY. Stony Brook University M.S. Thesis, 51 pages.

Young, Caitlin (2013) Fate of Nitrogen during Submarine Groundwater Discharge into Long Island North Shore Embayments. Stony Brook University Ph.D. Dissertation, 165 pages.

Report submitted by: Cornelia Schlenk, NYSG Date: June 30, 2014

NYSG Completion Report Instructions & Required Format

Report Written By: Darcy J. Lonsdale, Christopher J. Gobler, David Rawitz, and Laura M. Treible   Date: May 12, 2014

Portions of this report have been modified from the M.S. thesis by L.M. Treible (2013) or from a manuscript currently in review (Treible, Lonsdale, and Gobler) (see C6). Additional information is from the M.S. thesis in preparation by D. Rawitz (see D2 for each research focus).

A. Project Number and Title:R/CE-31-NYCT The Influence of Gelatinous Zooplankton on Nutrient Cycles, Hypoxia, and Food webs across Long Island Sound

B. Project Personnel: Darcy J. Lonsdale and Christopher J. Gobler, Project Investigators; Laura M. Treible and David Rawitz, NYSG Scholars; Lee Holt, technician.

C. Project Results:

C1. Meeting the Objectives:

Objective 1: Conduct field studies on the pelagic population cycles of gelatinous zooplankton

Field sampling began in May and continued through October in both 2011 and 2012. Bi-weekly sampling occurred at three stations in Long Island Sound: Western Long Island Sound (WLIS; 40˚52.320N, 73˚ 44.040W), Central Long Island Sound (CLIS; 41˚3.572 N, 73˚8.674 W), and a site in-between the two, the “middle” site (MLIS; 40˚59.085N, 73˚27.038W). To determine abundances of gelatinous zooplankton, oblique net tows were performed at each station with 202-µm and 1000-µm mesh nets fitted with a flowmeter (n=2 for each). Tows were conducted for 2-4 minutes to minimize net clogging or damage to gelatinous zooplankton. Even though other gelatinous zooplankton species occur in Long Island Sound, only the ctenophore *Mnemiopsis leidyi* was collected during the sampling period. All ctenophores were placed into a graduated cylinder to measure the total biovolume (ml) for each tow. Each ctenophore in the sample was measured for length with a graduated petri dish to the nearest tenth of a cm. If the total biovolume greatly exceeded 500 ml, a subsample (400-500ml) was taken from the well-mixed sample to perform the counts and measurements. Abundance (ind. m^{-3}) and biovolume (mL m^{-3}) were calculated by combining the individual gelatinous zooplankton counts (individuals) and biovolumes (mL) with the calculated volume of seawater sampled (m^{-3}).

In 2011, ctenophore biovolume was below 1.0 mL m^{-3} in May and early June (Figure 1). The population peak biomass occurred mid-July to early August, reaching 71.2 ± 17.5, 35.4 ± 10.4, and 53.4 ± 8.5 mL m^{-3} for WLIS, MLIS, and CLIS, respectively. Ctenophore biovolume began to decline mid-September and remained low for the rest of the sampling season. Biovolumes of *M. leidyi* were markedly lower in 2012, remaining below 1 mL m^{-3} at WLIS throughout the sampling season (Figure 2). Minor increases in biovolume occurred at MLIS and CLIS in early July and again at CLIS in late August (7.49 ± 1.69 mL m^{-3}), but biovolumes remained below 3 mL m^{-3} at MLIS and CLIS for the rest of the sampling dates.

Objective 2: Quantify ingestion rates and mortality impacts of gelatinous zooplanktivores on mesozooplankton and micrometazoa

In order to determine ingestion and mortality rates of ctenophores on zooplankton additional oblique tows were performed at each site to determine mesozooplankton and micrometazoan composition and abundance. In 2011, net tows were performed with a 0.5-m diameter, 64-µm mesh net (n=2) and in 2012 additional tows were performed using a 202-µm mesh net (n = 2) for comparison with previous studies of zooplankton in LIS (e.g., Capriulo et al. 2002). Upon completion of each tow, contents of the cod end were rinsed onto a 64-µm sieve and preserved. Zooplankton samples were identified and enumerated to the lowest taxonomic level.

In 2011, coincident with increases in adult ctenophores, copepod abundance decreased, which in turn may have released microplankton from predation pressure as the latter’s numbers increased indicating a trophic cascade

(Figures 3a-c). Such population relationships have been previously documented in Great South Bay (McNamara et al., 2013). Preliminary results from cross-correlation analysis indicated a positive correlation between adult *M. leidyi* and microplankton at the three sites. In contrast to 2011, there was no correlation between the above-described abundances in 2012 (Fig. 3d-f). In 2012, ctenophore predation on copepods was significantly lower than in 2011 (see below), hence ctenophore adult and microplankton populations did not demonstrate any correlations.

Estimates of ctenophore consumption rates (based on gut-contents and estimates of digestion time) of copepods highlight the interannual variation of the impact that ctenophores on zooplankton in LIS. In 2011 the consumption rate of copepods by the ctenophore population averaged ~ 3,117 m^{-3} d^{-1} in the WLIS and 5,308 m^{-3} d^{-1} in the CLIS, while in 2012, the average rates were only 8.0 m^{-3} d^{-1} and 35.5 m^{-3} d^{-1}, respectively. The predatory impacts of ctenophores on the net growth rate of prey populations were also variable between stations and prey species. For example, the daily predation rates on the copepods *Acartia tonsa* and *Oithona similis*. were compared to estimates of their daily population growth (the latter data obtained from Huntley and Lopez, 1992). In CLIS in 2011, *M. leidyi* may have consumed up to 67.2% of the daily growth of adults and copepodites of *A. tonsa*, 13.5% of their nauplii but only 2.6% of *O. similis* adults and copepodites. In the WLIS, the highest estimated ctenophore consumption was 13.2% of the daily growth of *A. tonsa*, 5.6% of nauplii, and 6.8 % of *O. similis*.

Objective 3: Estimate the oxygen consumption rates of gelatinous zooplankton

Given the substantial lack of ctenophore consumers in LIS, the probable fate of the dead ctenophores is bacterial decomposition, and thus the collapse of a ctenophore bloom could potentially promote localized hypoxic events. Using the carbon decomposition rates for dead ctenophores (see below), and assuming an elemental ratio of 138 O_2: 106 C, when ctenophores died, bacteria could have depleted 270.1 μmol O_2 m^{-3} day^{-1} in WLIS and 28.4 μmol O_2 m^{-3} day^{-1} in CLIS. In LIS, total bacterial respiration ranges from 0.3 to 1.5 mmol O_2 m^{-3} $hour^{-1}$ (Goebel and Kremer 2006). Thus, in the Western Sound, bacterial respiration from decaying ctenophore biomass would represent less than 5% of total bacterial respiration, and in the Central Sound this contribution would be even less. Although these numbers are relatively small percentages in relation to overall bacterial respiration, any additional oxygen depletion from decaying ctenophores could contribute to the summer hypoxia that occurs annually in LIS.

Ctenophores may also contribute to oxygen depletion in the LIS *via* their metabolism and respiration. The data on carbon content of ctenophores is being utilized to estimate their individual respiration rates (ml O_2 d^{-1}). The conversion of carbon content to respiration rate (RR) according to Purcell (2009) is as follows: $Log_{10}RR$ (ml O_2 d^{-1}) = 0.871 * Log_{10} (g C) + 1.686. Total oxygen consumption by larval (< 0.5 cm) and adult ctenophores (mL O_2 m^{-3} d^{-1}) will then be determined based on total abundance. At the present time, the data are still being worked up.

Objective 4: Quantify the reservoir of nutrients held in gelatinous zooplankton and nutrient-release rates of living and decomposing animals

<u>Reservoir of nutrients held by ctenophores</u>

Elemental analysis was performed in triplicate on a variety of size classes of *M. leidyi* in 2011. Ctenophores were collected from WLIS from June 21 – August 17 (n=5 dates), and from CLIS from June 16 – September 13 (n=8 dates) for elemental analysis. (Due to the low field abundances of adult organisms throughout 2012, no sampling dates provided sufficient numbers to collect ctenophores for elemental analysis or nutrient-release experiments). Individual ctenophores were measured and weighed (wet-weight). Dry-weights were determined after drying for 24 hours at 60°C. Each dried sample was then stored in tin foil packages for elemental analysis at a later date. Subsequently, each sample was homogenized using a mortar and pestle. Samples were analyzed for particulate carbon and nitrogen content. The elemental content of individuals (mg ind^{-1}) was normalized to dry weight (mg gDW^{-1}). A relationship was determined ($DW = 0.0074(length)^{2.13}$) between length and dry weight which allowed for estimation of total field population dry weight (gDW m^{-3}) from measured ctenophore size distribution. The elemental analysis results (mg gDW^{-1}) were combined with the field population data (gDW m^{-3}) to determine the total pool of carbon and nitrogen held in the LIS populations (mg m^{-3}). These values from the peak of the bloom (Aug. 3 in WLIS and Jul. 19 in CLIS) were also used to determine quantities of nitrogen and carbon released back into the system upon demise of the bloom.

The elemental composition of ctenophores averaged 19.4 ± 5.4 mgC gDW^{-1} and 4.0 ± 1.1 mgN gDW^{-1} in WLIS and 17.4 ± 4.3 mgC gDW^{-1} and 3.7 ± 0.9 mgN gDW^{-1} in CLIS (Fig. 4). Carbon:nitrogen ratios of ctenophores were significantly different between WLIS and CLIS (nested ANOVA, date within site; df=2,136, F=3.84, $p<0.05$), and at both sites, C:N ratio significantly decreased over the sampling season (df=4,136, F=5.65, $p<0.001$). There was no relationship between C:N ratios and ctenophore size.

After combining elemental analysis results with biomass estimates, at the highest biovolume of ctenophores in WLIS in 2011 (August 3), the population sequestered about 2913 μmol C m^{-3} and 580 μmol N m^{-3}. The CLIS had the largest biovolume in mid-July, and contained 1291 μmol C m^{-3} and 239 μmol N m^{-3}. The ctenophore post-bloom occurred in mid-August and September at WLIS and CLIS, respectively. At this time, in WLIS, ctenophores held only ~8 μmol C m^{-3} and 1.6 μmol N m^{-3}. The population decline at WLIS was abrupt (14 d) indicating an average biomass loss of 207 μmol C m^{-3} day^{-1} and 41 μmol N m^{-3} day^{-1}. In CLIS, post-bloom populations held 69 μmol C m^{-3} and 14 μmol N m^{-3}. The decline in the population from peak biovolumes at CLIS occurred gradually over 56 days. Although rates of biomass loss and decomposition would generally slow as the ctenophore population declined, average rates of biomass loss would be about 22 μmol C m^{-3} day^{-1} and 4 μmol N m^{-3} day^{-1}.

<u>Nutrient-release impacts</u>

In 2011, ctenophores were collected from WLIS from July 6 – August 17 (n=3 dates), and from CLIS from July 6 – August 8 (n=4 dates) for nutrient-release experiments. Nutrient-release experiments were performed on live organisms, in triplicate, on a variety of size classes. Individual organisms were placed in 1.2-L containers containing 0.2-μm filtered seawater collected from the sampling stations. Initial dissolved nutrient samples were obtained as previously described, prior to starting each experiment. Containers were incubated in the dark at ambient seawater temperature for 12 hours. Dissolved nutrient samples (10ml, n=2) were obtained from each container every three hours for a total of 12 hours. After the 12-h incubation, the wet-weights of each individual ctenophore were recorded. Dry-weights were determined after drying for 24 hours at 60°C. Dissolved nutrient analyses were performed for NH_4^+ and PO_4^{-3} concentration for all experimental samples.

In 2011, ctenophores in WLIS released ammonium at rates from about 0.006 to 0.62 μmol ind^{-1} h^{-1}, and phosphate at rates from about 0.004 to 0.13 μmol ind^{-1} h^{-1}. Release rates of both ammonium and phosphate were significantly dependent on size (df=1,50, F=42.712, $p<0.001$; df=1,45, F=8.4021, $p<0.01$). Temporal dynamics of seawater temperature and mesozooplankton densities and their relationship to release rates were also investigated. The combined effect of temperature and ctenophore weight more accurately described the nutrient-release rates than the combined effect of weight, temperature, and food concentration or weight alone.

In WLIS, the maximum turnover of both ammonium and phosphate by the ctenophore population likely occurred on August 3, when its biovolume was at a maximum. Using a rate to body weight relationship where $Rate = a\, DW^b$ and *Rate* is the nutrient release rate in μmol ind^{-1} h^{-1}, DW the dry weight of the individual in grams, and *a*, and *b* are constants used to fit the relationship to the data, the total population of *M. leidyi* at peak abundance could have released ammonium and phosphate of up to 49 μmol m^{-3} day^{-1} and 13 μmol m^{-3} day^{-1}, respectively. Comparing these rates to nutrient pools in WLIS (data not presented), the total population could have turned over up to 3.20% day^{-1} of ammonium and 0.57% day^{-1} of phosphate.

In CLIS, maximum daily population release of ammonium and phosphate by ctenophores would have occurred on July 6 when the total *M. leidyi* population could have released ammonium and phosphate of up to 37.17 μmol m^{-3} day^{-1} and 15.55 μmol m^{-3} day^{-1}, respectively. Scaled to population abundance, the contribution of ctenophores to the total pool of inorganic nutrients in LIS was estimated to range between 3.20-13.76% day^{-1} of ammonium and 0.57-3.88% day^{-1} of phosphate, depending on site. In LIS in the summer, nitrogen pools are turned over several times daily by phytoplankton. It would take peak abundances of *M. leidyi* in WLIS over 31 days to turn over the ammonium pool at that site. Due to lower stocks of nitrogen in the CLIS, it would take about 7 days for the maximum abundance of CLIS ctenophores to turn over the ammonium pool. These turnover rates were significantly lower than rates in Chesapeake Bay and Narragansett Bay (Condon et al. 2010), possibly due to differences in bloom biomass and other nutrient inputs.

Estimating an average rate of primary production in the summer from oxygen evolution (430 mmol O_2 m^{-2} d^{-1}; Goebel et al. 2006), an oxygen to carbon ratio of 138 O_2:106 C, and assuming that the elemental composition of phytoplankton conformed to the Redfield ratio of 106 C:16 N:1 P, the amounts of ammonium and phosphate released by peak *M. leidyi* populations could support <1% of daily primary production in LIS (Schneider 1989).

Objective 5: Model the ecosystem-wide impacts of gelatinous zooplankton on nutrient cycles, hypoxia, and food webs across LIS.

Portions of this objective have been addressed (see the above regarding evidence for a trophic cascade and nutrient turnover rates) while the contribution of ctenophores to hypoxia has yet to be thoroughly addressed.

C2. Scientific Abstract: In most marine environments, gelatinous zooplankton play a significant role in the food web dynamics of the water column. The “top-down” influences of gelatinous zooplankton have been the primary focus of many studies, but there is increasing evidence they may also have important “bottom-up” impacts on primary productivity through nutrient sequestration and/or excretion. We studied both these aspects of the ctenophore *Mnemiopsis leidyi*, the most abundant gelatinous zooplankton in Long Island Sound (LIS) at three stations from the central basin to the western basin during 2011-2012. One striking aspect of this study was the large interannual variation in ctenophore abundance with 2011 experiencing relatively high abundances while in 2012 the peak abundances were on the order of a magnitude lower. In 2011, ctenophore and microplankton abundances were positively correlated, suggesting a trophic cascade brought about by the reduction of mesozooplankton from ctenophore predation. The predatory impacts of ctenophores on their prey, such as copepods, were also variable between stations and among prey. For example, the daily predation rates by the ctenophore population were compared to estimates of daily population growth for the copepods *Acartia tonsa* and *Oithona similis* (the latter rates obtained from Huntley and Lopez, 1992). In 2011 in the central basin at peak ctenophore abundance, *M. leidyi* may have consumed up to 67.2% of the daily production of adults and copepodites of *A. tonsa*, 13.5% of their nauplii but only between 0% to 2.6% of *O. similis* adults and copepodites. In the WLIS, the highest estimated rate of ctenophore consumption was lower for *A. tonsa* (13.2%) and nauplii (5.6%) but 6.8 % for *O. similis.*

In 2011, to address the “bottom-up” role of ctenophores, the chemical content and nutrient remineralization rates (i.e., NH_4^+, PO_4^{3-}) of *M. leidyi* were quantified. Ctenophores remineralized NH_4^+ and PO_4^{3-} at rates up to 0.62 μmol ind^{-1} h^{-1} and 0.13 μmol ind^{-1} h^{-1}, respectively, and were capable of large releases of nutrients upon the demise of the bloom (48.59 μmol m^{-3} day^{-1} of NH_4^+ and 13.41 μmol m^{-3} day^{-1} of PO_4^{3-}). However in both cases, these rates were not in quantities sufficient to support a sizeable fraction of primary production (< 1 % d^{-1}) when compared to the background concentrations of the nutrients. Collectively, this study suggests that gelatinous zooplankton may make only a minor contribution to nutrient pools in highly eutrophic, urban estuaries.

Given the substantial lack of ctenophore consumers in LIS, the likely fate of the dead ctenophores is bacterial decomposition, and thus the collapse of a ctenophore bloom could potentially exacerbate localized hypoxic

events. Using the carbon decomposition rates for dead ctenophores (207.5 μmol C m^{-3} day^{-1} in WLIS and 21.8 μmol C m^{-3} day^{-1} in CLIS), and the elemental ratio of 138 O_2: 106 C, when ctenophores died, bacteria could deplete 270.1 μmol O_2 m^{-3} day^{-1} in WLIS and 28.4 μmol O_2 m^{-3} day^{-1} in CLIS. In LIS, total bacterial respiration ranges from 0.3 to 1.5 mmol O_2 m^{-3} $hour^{-1}$ (Goebel and Kremer 2006). Thus, in the Western Sound, bacterial respiration from decaying ctenophore biomass would represent less than 5% of total bacterial respiration, and in the Central Sound this contribution would be even less. Although these numbers are relatively small percentages in relation to overall bacterial respiration, any additional oxygen depletion from decaying ctenophores could contribute to the summer hypoxia that occurs annually in LIS.

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Capriulo, G.M., G. Smith et al. (2002) The planktonic food web structure of a temperate zone estuary, and its alteration due to eutrophication. Hydrobiologia 475/476: 263-333.

Goebel N.L., J.N. Kremer, C.A. Edwards (2006) Primary production in Long Island sound. Estuaries and Coasts 29: 232-245.

Huntley, M.E., M.D.G. Lopez (1992) Temperature-dependent production of marine copepods: A global synthesis. American Naturalist 140: 201-242.

McNamara, M.E., D.J. Lonsdale, R.M. Cerrato (2013) Top-down control of mesozooplankton by adult *Mnemiopsis leidyi* influences microplankton abundance and composition enhancing prey conditions for larval ctenophores. Estuarine, Coastal and Shelf Science 133: 2-10

C3. Problems Encountered: None

C4. New Research Directions: The research project remained essentially the same as proposed.

C5. Interactions:

1. Dr. James P. Browne, Conservation Biologist for the Town of Hempstead, Department of Conservation and Waterways contacted us regarding this study as he has been looking into the correlations between nutrients and various marine species, including ctenophores, on the south shore and north shores of Long Island, including Long Island Sound. He was sent a PDF of Ms. Treible's thesis.

2. Dr. Thomas F. Ihde, Fisheries/Ecosystem Modeler with the National Marine Fisheries Service, NOAA Chesapeake Bay Office also requested information on our project and was interested in the estimates of nutrient regeneration by ctenophores. He also was sent Ms. Treible's thesis.

C6. Presentations and Publications:

Presentations:

1. Treible, L.M., D.J. Lonsdale, and C.J. Gobler, Long Island Sound Research Conference, "The role of ctenophores in nutrient regeneration in Long Island Sound", Port Jefferson, NY, 2013.
Abstract: Gelatinous zooplankton blooms have been increasing in magnitude and frequency globally. Seasonal variations in food availability and temperature can trigger a population bloom and subsequent crash in coastal and estuarine waters. Long Island Sound (LIS) is a highly-productive urban estuary. Due to its proximity to New York City and annual summer hypoxia, there has been substantial focus on anthropogenic nutrient inputs and reductions to LIS. When determining nutrient budgets, an important process is the recycling of nutrients within a system. Gelatinous zooplankton, including the most common species in LIS, the ctenophore *Mnemiopsis leidyi*, are capable of significant rates of nutrient regeneration. During 2011, the population biomass of *M. leidyi* was monitored and nutrient regeneration rates (i.e., NO_3^-, PO_4^{3-}) were calculated based on laboratory experiments.

Preliminary results from 2011 indicate that ctenophores in LIS have the potential, at times, to overturn substantial amounts of these nutrients daily. However, in 2012, the ctenophore bloom never occurred suggesting that rates of nutrient regeneration by gelatinous zooplankton also can vary widely on an interannual basis, complicating the assessment of the nutrient budget for LIS.

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2. Treible, L.M., D.J. Lonsdale, and C.J. Gobler, Aquatic Sciences Meeting, "The role of ctenophores in nutrient regeneration in Long Island Sound", New Orleans, LA, 2013
Abstract: Gelatinous zooplankton blooms have been increasing in magnitude and frequency globally. Seasonal variations in food availability and temperature can trigger a population bloom and subsequent crash in coastal and estuarine waters. Long Island Sound (LIS) is a highly-productive urban estuary. Due to its proximity to New York City and annual summer hypoxia, there has been substantial focus on anthropogenic nutrient inputs and reductions to LIS. When determining nutrient budgets, an important process is the recycling of nutrients within a system. Gelatinous zooplankton, including the most common species in LIS, the ctenophore *Mnemiopsis leidyi*, are capable of significant rates of nutrient regeneration. During 2011, the population biomass of *M. leidyi* was monitored and nutrient regeneration rates (i.e., NO_3^-, PO_4^{3-}) were calculated based on laboratory experiments. Preliminary results from 2011 indicate that ctenophores in LIS have the potential, at times, to overturn substantial amounts of these nutrients daily. However, in 2012, the ctenophore bloom never occurred suggesting that rates of nutrient regeneration by gelatinous zooplankton also can vary widely on an interannual basis, complicating the assessment of the nutrient budget for LIS.

3. Treible, L.M., D.J. Lonsdale, and C.J. Gobler. The Role of Ctenophores in Nutrient Regeneration in Long Island Sound, Long Island Marine Habitat (MAR 303-S) Graduate Student Presentations, Southampton, NY

Other Outreach:

Treible, L.M. Center for Talented Youth (CTY) Science and Technology Series; led the students in plankton tows and showed them various species under the microscope, 2012

Treible, L.M., D.J. Lonsdale, D. Rawitz. "VIP" Cruise on the R/V Seawolf Stony Brook University President, members of the President's council, and News 12 Network owner; performed plankton tows and showed them organisms under a microscope

Manuscript Submitted:

Treible, L.M., D.J. Lonsdale, and C.J. Gobler. The role of ctenophores in nutrient regeneration in Long Island Sound. Mar. Ecol. Prog. Ser. (in revision)

D. Accomplishments:

D1. Impacts & Effects: This study has addressed some fundamental data gaps on the role of ctenophores in the planktonic food web in LIS, including the predatory impact of ctenophores on zooplankton and nutrient cycling, including nitrogen. Nitrogen has been the focus of numerous experimental and modeling efforts as it relates to hypoxia in the Long Island Sound. The data generated by our research may also be of interest to others studying the ecology of this ubiquitous group of marine planktonic predators. Numerous published studies have indicated that gelatinous zooplankton may have a substantial impact on rates of primary productivity via nutrient regeneration. Our study, albeit based on limited interannual sampling, concluded differently, in that in LIS gelatinous zooplankton make only a minor contribution to nutrient pools in this eutrophic estuary. We anticipate that the results of this study will also useful for resource managers and ecosystem modelers in that it will provide information on the potential contribution of ctenophores to community oxygen demand generated by the living ctenophores and bacterial decomposition of decaying ctenophore biomass. As described above, we have already received requests for our data from a resource manager and an ecosystem modeler.

D2. Scholar(s) & Student(s) Status:

1. Laura M. Treible, M.S. degree May 2013
   "The Role of the Ctenophore *Mnemiopsis leidyi* on Nutrient Cycling in Long Island Sound, NY", Stony Brook University, 40 p.

   Current employment: Instructor, University of North Carolina Wilmington, undergraduate biology laboratory (BIO105), and laboratory technician for Rob Condon, a gelatinous zooplankton ecologist. Laura will also be pursuing her Ph.D. this fall at UNC in this area of study.

2. David Rawitz, M.S. degree expected August 2014
   "On the Impacts of the Lobate Ctenophore *Mnemiopsis leidyi* to Zooplankton Community Dynamics in the Long Island Sound"

D3. Volunteers:

Lauren VanSicklin, undergraduate from Delaware Valley College, assisted in the field and laboratory between May and Nov 2012.

D4. Patents: None

E. Stakeholder Summary:

In most marine environments, gelatinous zooplankton play a significant role in the food web dynamics of the water column. Large population increases and subsequent crashes of these animals are common in coastal waters. Gelatinous zooplankton, including ctenophores, are effective top predators and their voracious feeding has consequences that can cascade through the planktonic food web. And although this "top-down" impact of gelatinous zooplankton has been the primary focus of many studies, there is increasing evidence they may also have an important "bottom-up" impact on primary productivity through nutrient sequestration and/or excretion. We studied both these aspects of the ctenophore *Mnemiopsis leidyi*, the most abundant gelatinous zooplankton in Long Island Sound (LIS) during 2011-2012. One striking aspect of this study was the large interannual variation in ctenophore abundance with 2011 experiencing relatively high abundances while in 2012 the peak abundances were an order of a magnitude lower than the previous year. In turn, the predatory impacts on their prey, such as copepods, were also highly variable between the two years.

In 2011, to address the "bottom-up" role of ctenophores in LIS we quantified the chemical content of the ctenophore population and their excretion rates of nutrients required by phytoplankton (i.e., nitrogen and phosphate). The chemical content of the ctenophores indicates how much of various nutrients can be released back into the environment upon population demise while excretion rates measures ctenophore effects on nutrient cycles while the population is thriving. Our findings indicated that neither process provided nutrients in sufficient quantities to support a significant fraction of primary production in LIS ($< 1\%\ d^{-1}$) when compared to the background concentrations of nutrients. Collectively, this study suggests that gelatinous zooplankton may make only a minor contribution to nutrient pools in highly eutrophic estuaries such as LIS.

F. Pictorial: Previously provided to the New York Sea Grant Institute.

Figures

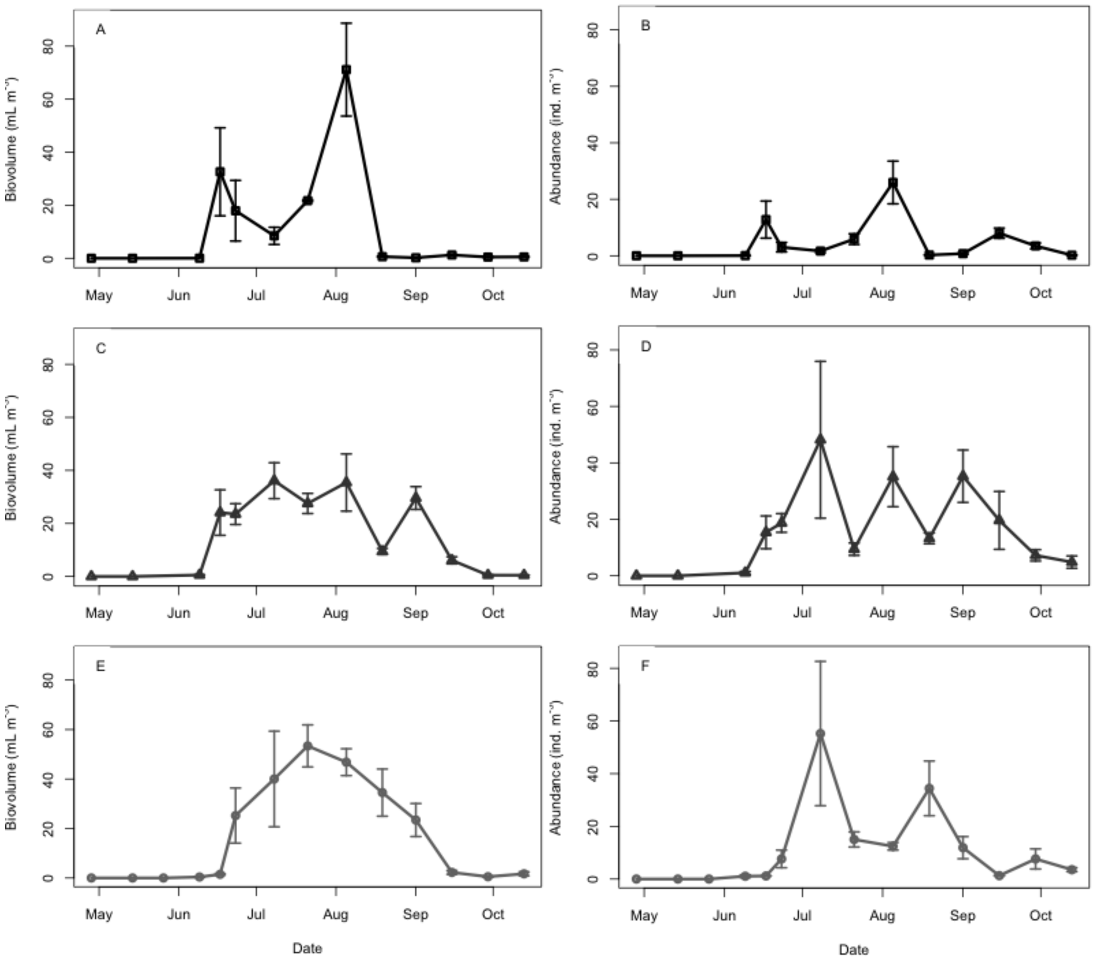


Figure 1**.** Ctenophore biovolumes (±1 s.e.; mL m^{-3}; A, C, E) and abundances (±1 s.e.; ind. m^{-3}; B, D, F) at WLIS (A, B), MLIS (C, D), and CLIS (E, F) during 2011.

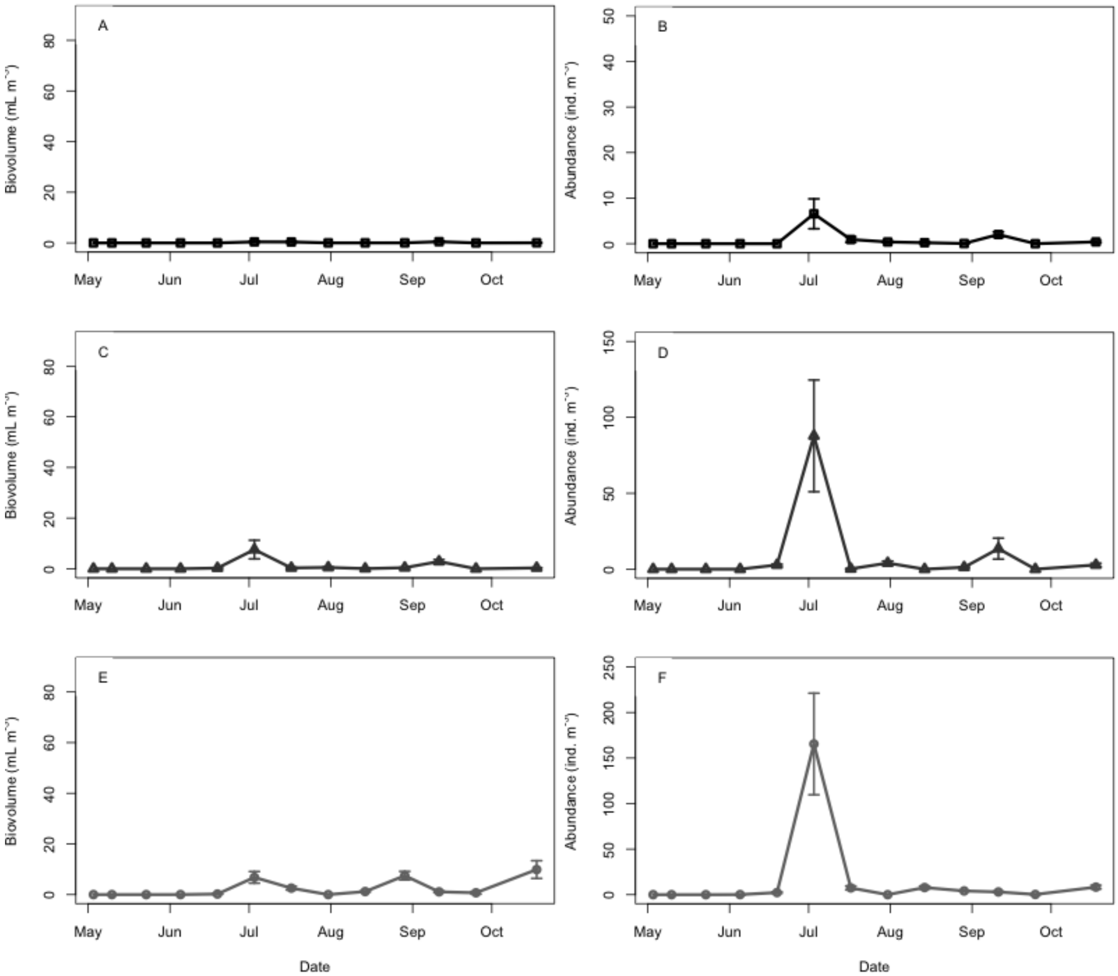
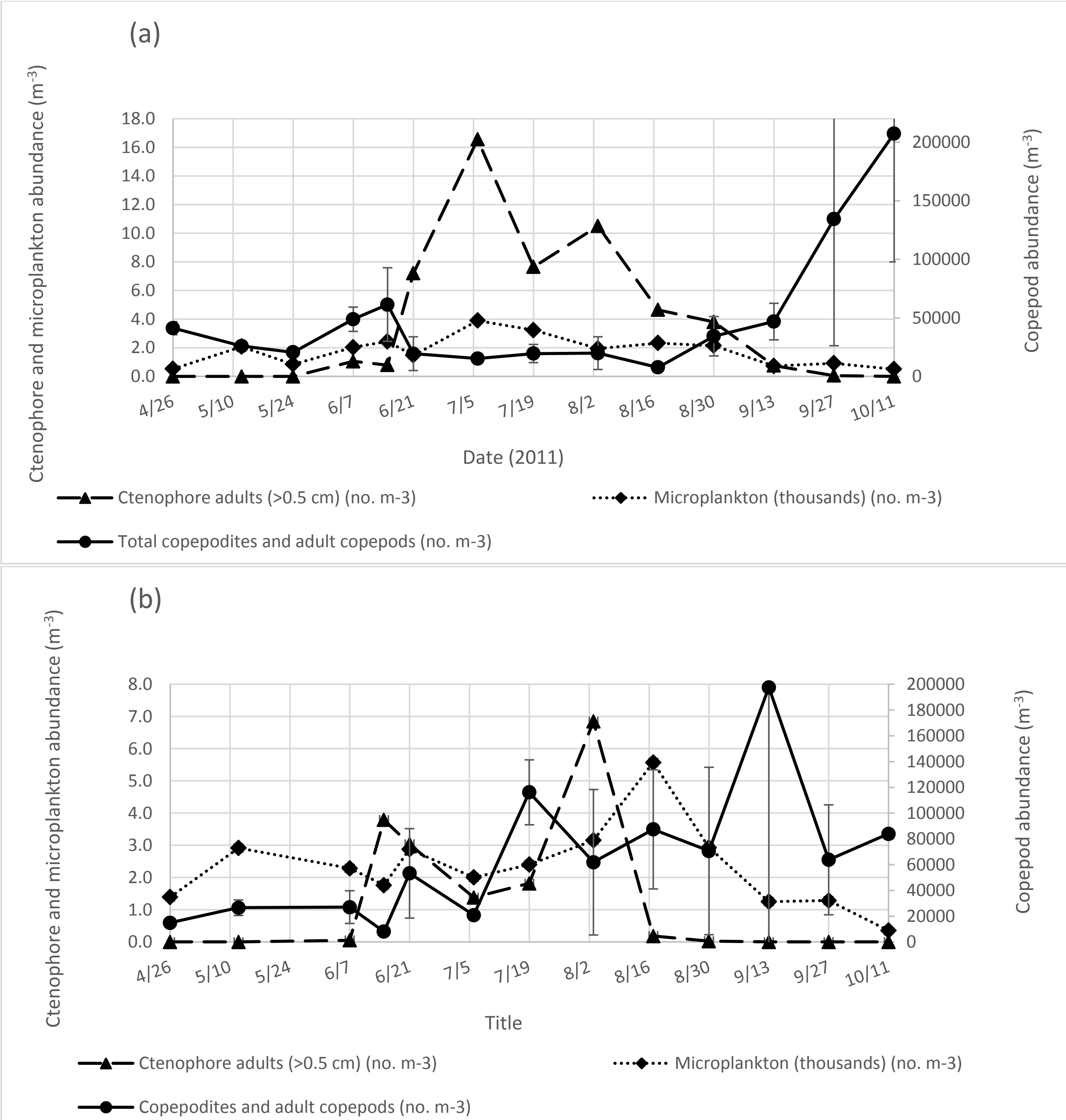
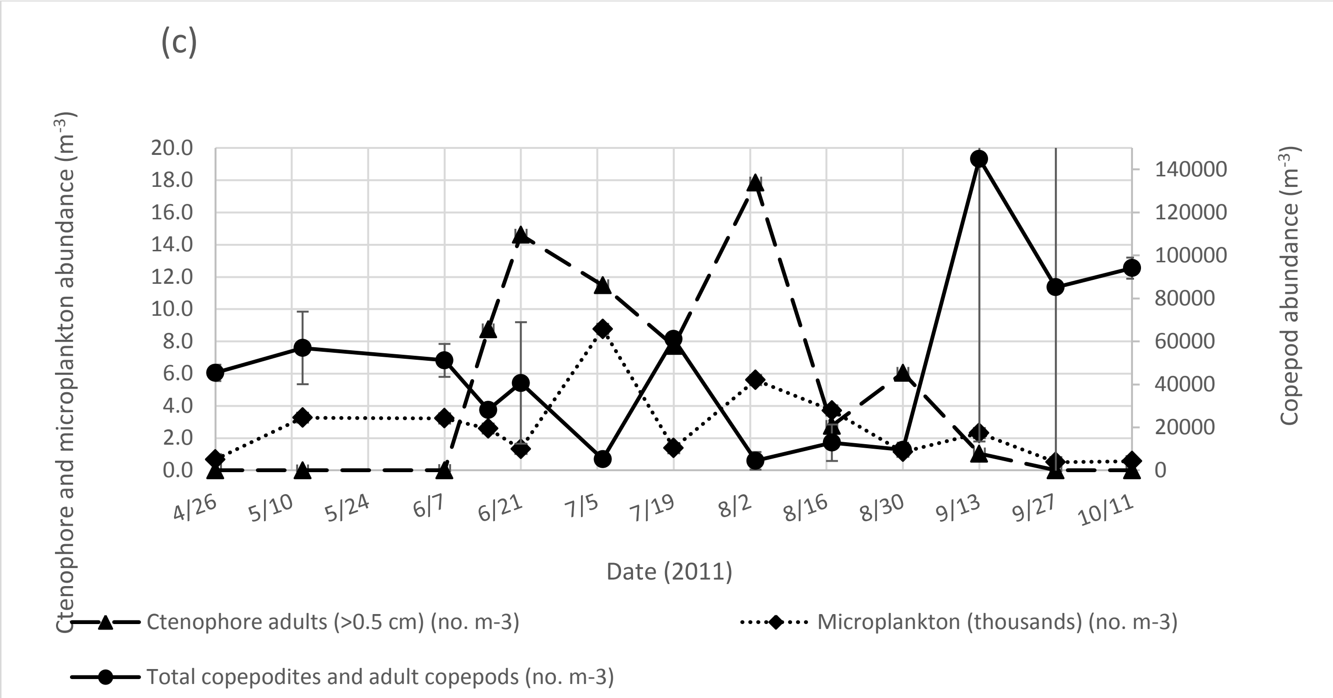


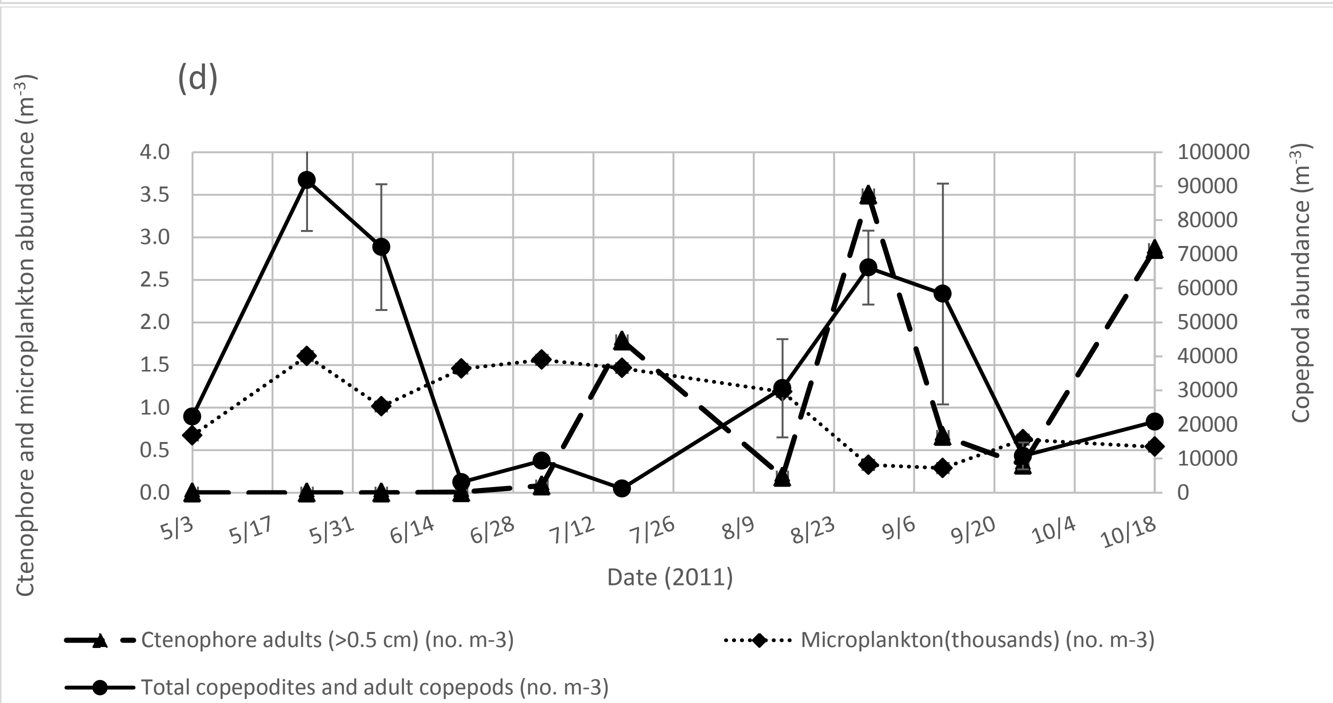
Figure 2. 2012 Ctenophore biovolumes (±1 s.e.; mL m^{-3}; A, C, E) and abundances (±1 s.e.; ind. m^{-3}; B, D, F) at WLIS (A, B), MLIS (C, D), and CLIS (E, F) during 2012.

Figure 3. Seasonal abundances of adult ctenophores, microplankton, and total copepodites and adult copepods (no. m^{-3}) in (a) CLIS-2011, (b) WLIS-2011, (c) MLIS-2011, (d) CLIS-2012 (e) WLIS-2012, (f) MLIS-2012.

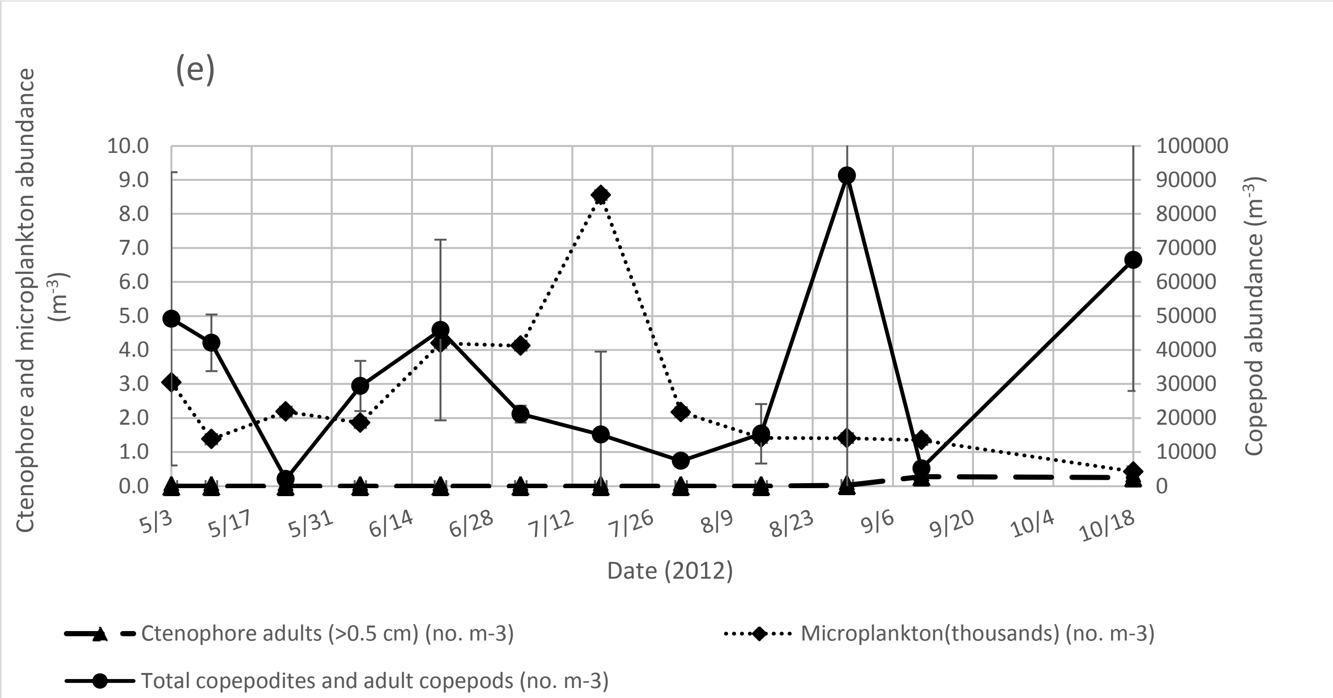




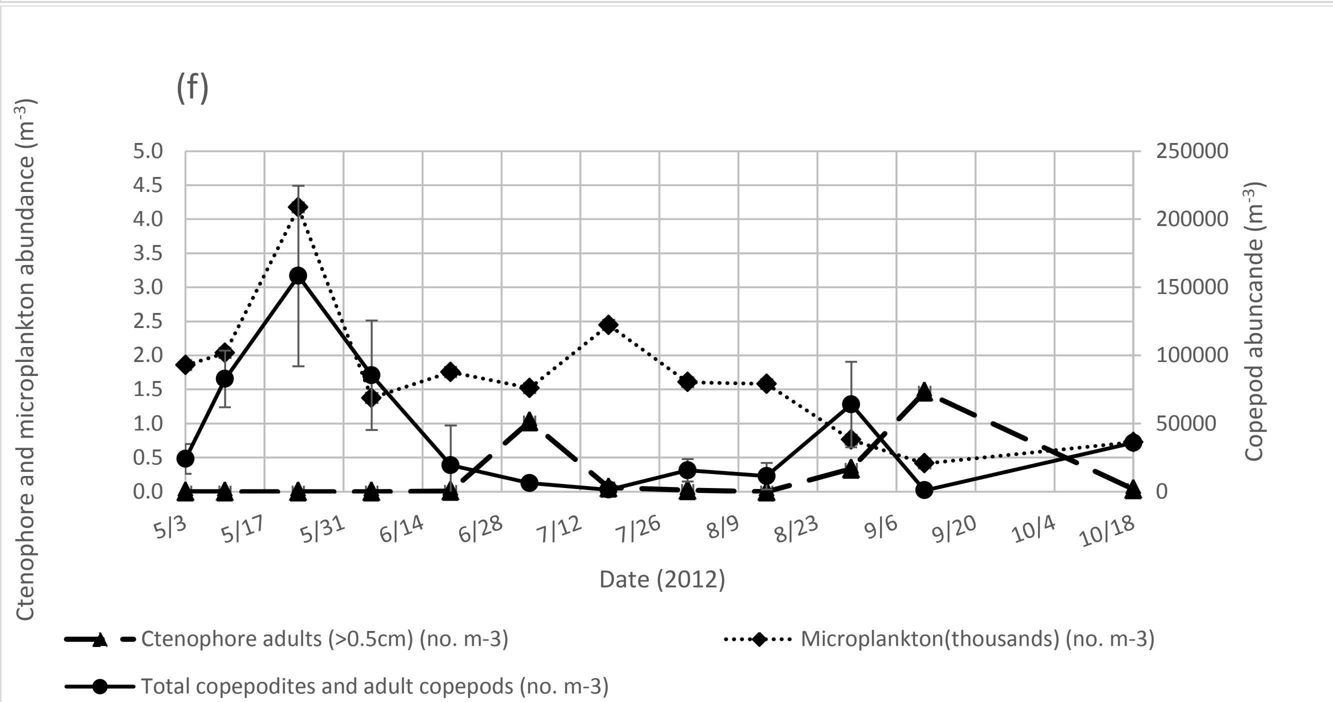
(c)
Ctenophore and microplankton abundance (m-3)
20.0
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
140000
120000
100000
80000
60000
40000
20000
0
Copepod abundance (m-3)
4/26
5/10
5/24
6/7
6/21
7/5
7/19
8/2
8/16
8/30
9/13
9/27
10/11
Date (2011)
Ctenophore adults (>0.5 cm) (no. m-3)
Microplankton (thousands) (no. m-3)
Total copepodites and adult copepods (no. m-3)



(d)
Ctenophore and microplankton abundance (m-3)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
Copepod abundance (m-3)
5/3
5/17
5/31
6/14
6/28
7/12
7/26
8/9
8/23
9/6
9/20
10/4
10/18
Date (2011)
Ctenophore adults (>0.5 cm) (no. m-3)
Microplankton(thousands) (no. m-3)
Total copepodites and adult copepods (no. m-3)



(e)
Ctenophore and microplankton abundance (m-3)
Copepod abundance (m-3)
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
5/3
5/17
5/31
6/14
6/28
7/12
7/26
8/9
8/23
9/6
9/20
10/4
10/18
Date (2012)
Ctenophore adults (>0.5 cm) (no. m-3)
Microplankton(thousands) (no. m-3)
Total copepodites and adult copepods (no. m-3)



(f)
Ctenophore and microplankton abundance (m-3)
Copepod abuncande (m-3)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
250000
200000
150000
100000
50000
0
5/3
5/17
5/31
6/14
6/28
7/12
7/26
8/9
8/23
9/6
9/20
10/4
10/18
Date (2012)
Ctenophore adults (>0.5cm) (no. m-3)
Microplankton(thousands) (no. m-3)
Total copepodites and adult copepods (no. m-3)

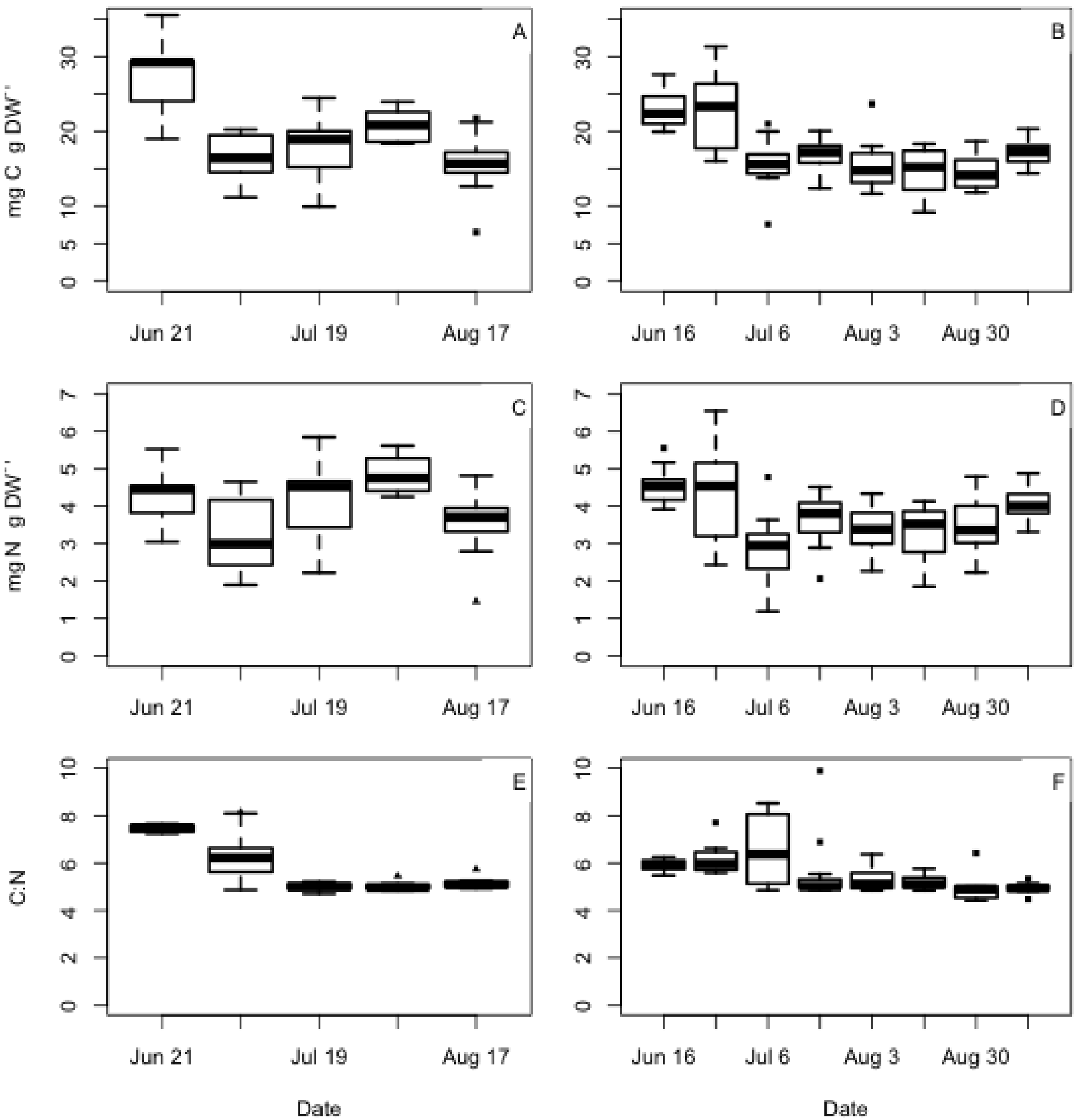


Figure 4. Ctenophore body composition (mgC gDW^{-1} A, B; mgN gDW^{-1}, C, D) and body composition ratios (C:N; E, F) at WLIS (A, C, E) and CLIS (B, D, F) for the 2011 sampling season.

Report Written By: Chris Gobler and Theresa Hattenrath-Lehmann **Date:** MAY 2013

A. Project Number and Title:

R/CMB-38-NYCT, Phase shifts among primary producers within Long Island Sound: Will anthropogenic stressors continue to expand the niche of PSP- and DSP-producing dinoflagellate blooms?

B. Project Personnel:

Chris Gobler, Principal Investigator, Theresa Hattenrath-Lehmann, NYSG Scholar

Project Results:

Objective 1: **Establish the temporal dynamics of phytoplankton including the toxic dinoflagellates *Dinophysis acuminata* and *Alexandrium fundyense,* PSP-and DSP toxins, and environmental variables along transects from near shore to open water regions.**

Field sampling - During 2011 and 2012, field samples were collected on a weekly to twice-weekly basis from March through August. Samples were collected at our main site, Northport Harbor (site 2 & 8, Fig.1) which is within the southeastern portion of the Northport-Huntington Bay complex, located on the north shore of Long Island, NY, USA. Cruises were conducted across six sites (4, 8, 9, 10, 16, and LIS; Fig. 1) to assess the spatial extent of these blooms. Additionally, samples were taken weekly to biweekly at several embayments across Long Island both on the north and south shore as well as along the east end (Tables 1 & 2). At each station, a YSI© probe was used to record surface temperature, salinity and dissolved oxygen. Subsurface water (~0.25m) was collected and whole water samples were preserved in Lugol's iodine. *Dinophysis* cell densities were enumerated using a 1ml Sedgewick-Rafter slide under a compound microscope using both whole water samples and concentrated water samples preserved in Lugol's iodine. Concentrated water samples were made in the field by sieving 1 - 2L of Northport Bay water through either a 200 μm or 64 μm mesh (to eliminate large zooplankton) and then onto a 20 μm sieve that was backwashed into a 15ml centrifuge tube. Concentrates were made to increase the limit of detection as *Dinophysis* cell densities are often a relatively small portion of the total phytoplankton community and are therefore expressed as cells per L. Counts made on plankton concentrates were not significantly different from direct counts on whole water. *Alexandrium fundyense* cell densities were enumerated using a highly sensitive molecular probe developed by Anderson et al. (2005b) and described at length in Hattenrath et al. (2010). Briefly, aliquots of phytoplankton concentrates (formalin and then methanol preserved) were hybridized with an oligonucleotide probe specific for the NA1 North American ribotype *Alexandrium fundyense/catenella/tamarense* with Cy3 dye conjugated to the 5' end (5'-/5Cy3/AGT GCA ACA CTC CCA CCA-3'). Cells were enumerated using a Nikon epifluorescence microscope with a Cy3™ filter set (Anderson et al., 2005).

Toxins in phytoplankton concentrates- Several liters of seawater were pre-sieved through a 200 μm mesh (to eliminate large zooplankton) and subsequently concentrated on a 20 μm sieve and backwashed into 15ml centrifuge tubes. Samples were centrifuged at 3000 rpm for 11 minutes and the supernatant aspirated without disturbing the cell pellet. Cell pellets were kept frozen at -20ºC until further analysis.

Analysis of DSP toxins- Algal pellets were resuspended in a known volume of either 100% or 80% aqueous methanol, homogenized by vortex mixing and probe-sonicated (Branson 1450 sonicator) on ice at 30% power, followed by centrifugation at 3400 x g for 10 min. The methanolic supernatants were filtered with a 0.2 μm syringe filter in preparation for analysis. Samples were analyzed for the presence of DSP toxins using liquid chromatography (HP 1100 series HPLC; Agilent Technologies, Palo Alto, CA) coupled with tandem mass spectrometry (4000 QTRAP hybrid triple quadrupole/linear ion trap mass spectrometer; AB Sciex, Foster City, CA) using the method described by Gerssen et al. (2009) with modifications. LC separation was performed on X-Bridge™ C18 (150 × 3 mm, 5 μm) column, (Waters, Milford, MA) using a mobile phase of water (A) and acetonitrile/water (90:10, V/V) (B), both containing 6.7 mM ammonium hydroxide under gradient elution at a flow rate of 0.4 mL min^{-1} (linear gradient from 1min of 10% B to 90% B at 12 min, hold for 3 min, then return to 10% B at 17 min and hold for 4 min). The detection of DSP toxins by MS was achieved by multiple reaction monitoring (MRM) in negative ion mode for OA, DTX1, and DTX2 (for OA and DTX2 with MRM transitions of *m/z* 803.5 →113.1and 255.1, for DTX1 with MRM transitions of *m/z* 817.5 → 113.1 and 255.1), and in positive ion mode for PTX11, PTX2, and their isomers (for PTX11 and its isomers with MRM transitions of *m/z* 892.5 →213.1 and 839.5, for PTX2 and its isomers with MRM transitions of *m/z* 876.5 → 213.1 and 823.5). Certified standards of OA, DTX1, DTX2, and PTX2 were available for toxin determination from NRC (Halifax, Canada) and RIKILT (Institute of Food Safety, The Netherlands). No standards were available for PTX11 and its isomers and PTX2 isomers; their concentrations were calculated approximately using PTX2 standards. PTX11 and its isomers showed identical product ion spectra but different LC retention time and their product ion spectra matched those published (Suzuki et al., 2003). PTX2 and its isomers also showed identical product ion spectra but different LC retention time. As such, all PTX concentrations were combined and reported as total PTXs (herein referred to as PTX). The detection limit was about 0.5 pg of OA, 0.65 pg of DTX1, 0.4 pg of DTX2, and 0.25 pg of PTX2 on LC column. The majority of toxin samples presented herein were not subjected to alkaline hydrolysis and therefore represent free toxins (i.e. esterified toxins are not included) and are therefore lower than the total OA (Deeds et al., 2010). However, to determine if esters were present in phytoplankton concentrates select samples (the peak of the *Dinophysis* blooms for 2011) were hydrolyzed using the procedure described in the section of the analysis of DSP toxins in shellfish.

Analysis of DSP toxins in shellfish- During 2010 and 2011, netted bags containing the blue mussel, *Mytilus edulis*, collected from regions without DSP toxins were deployed in the Northport-Huntington Bay complex (S1-S7; Fig. 1 (stars)). Mussel bags were collected sporadically from each site and mussels were shucked and frozen until analysis. Similarly, native soft shell clams (*Mya arenaria*) and ribbed mussels (*Geukensia demissa*) from Northport Harbor were harvested sporadically during the months of April through July (2011), shucked, and frozen until analysis. Samples of shellfish were homogenized and extracted in three volumes of 100% methanol, followed by centrifugation at 3000 x *g* for 5 min. The methanolic supernatants were filtered with a 0.2 µm syringe filter in preparation for analysis. Samples extracts were analyzed as in the above section on analyses of DSP toxins. In addition to analyzing for free acids, samples were also subjected to alkaline hydrolysis for the determination of esterified toxins. A known volume of 2.5M sodium hydroxide solution was added to sample extract, placed in a water bath at 76ºC for 45 minutes, allowed to cool to room temperature, and then neutralized with a known volume of 2.5M hydrochloric acid solution (Mountfort et al., 2001). All DSP toxins were analyzed at NOAA's Marine Biotoxin Laboratory.

***Alexandrium, Dinophysis,* and their respective toxins in phytoplankton concentrates: 2011 and 2012-** During spring of 2011, *Alexandrium* densities reached ~26,000 cells L^{-1} with peak saxitoxin concentrations reaching 760 pmol STX eq. L^{-1} (Fig 2). The large and extended bloom in Northport and Huntington Bays caused both native and bioassay shellfish to accumulate saxitoxin to levels which were a threat to human health and resulted in the closure of ~10,000 acres of shellfish beds in this system for most of May and June. Following the *Alexandrium* bloom, a large *D. acuminata* bloom reaching ~ 1.3 million cells L^{-1} occurred in Northport Bay which to our knowledge is the largest bloom recorded in North America (Fig 3). Transects across the Northport-Huntington Bay complex in 2011 showed that the highest *Dinophysis* densities were confined to the back part of Northport Harbor (site 2) with lower densities (ranging from 14 to 1,700 cells L^{-1}) occurring in other regions (Fig. 3). Toxins known to cause DSP (okadaic acid and dinophysistoxins) were found in phytoplankton concentrates in addition to another co-occurring potentially harmful toxin group, the pectenotoxins. In general, PTX concentrations were usually the most abundant particulate toxin followed by esterified OA, esterified DTX1, free DTX1 and free OA (Fig. 4, inset). Among the DSP toxins, esterified OA, esterified DTX1, free OA and free DTX1 represented 66%, 26%, 1% and 7%, respectively, of the total (Fig. 4 inset). DTX2 was not detectable within these blooms. Maximal particulate toxin levels during this study occurred in 2011 and were as follows: total OA =188 pg mL^{-1}, total DTX1= 86 pg mL^{-1}, and PTX = 2,900 pg mL^{-1}, free OA = 4.2 pg mL^{-1}, free DTX1 = 20.4 pg mL^{-1}, esterified OA = 185 pg mL^{-1} and esterified DTX1 = 66 pg mL^{-1} (Fig 4).

During spring of 2012, *Alexandrium* densities at Britannia (site 2) and Woodbine (site 8) marinas, both sites located in Northport Harbor, reached 23,000 and 11,000 cells L^{-1}, respectively (Fig. 5). This Northport bloom both started (15 March) and peaked earlier (7 May) than most previous year's blooms likely due to the unusually warm March temperatures experienced during 2012. Additional *Alexandrium* blooms occurred on the east end and south shore of Long Island in embayments such as Meetinghouse Creek (17,200 cells L^{-1}), Reeves Bay (3,000 cells L^{-1}), Mattituck (2,500 cells L^{-1}), Sag Harbor Cove (3,500 cells L^{-1}), and Weesuck Creek (300 cells L^{-1}; Fig. 6 and 7). During 2012, we detected PSP-producing *Alexandrium* at several other sites around Long Island at densities <100 cells L^{-1} (Table 1; Fig. 8). Overall, in 2012 *Alexandrium* was observed at 85% of the sites sampled, with 27% of those sites having densities of >1,000 cells L^{-1}.

Several locations were closed to shellfish harvest due to the presence of PSP contaminated shellfish (Fig. 9). On May 2nd Northport, Centerport and Duck Island Harbors as well as Northport Bay were closed to shellfish harvest. On May 16th these closures which lasted for approximately one month (closure rescinded June 8th) were expanded to Huntington Bay, Huntington Harbor and Lloyd Harbor. These Northport-Huntington Bay complex closures occurred weeks earlier than previous year's closures due to the earlier and extended *Alexandrium* bloom which was potentially caused by the unusually warm March. Additionally, approximately 92 acres of shellfish beds in Mattituck Creek and Mattituck Inlet were closed as of April 3rd 2012. Approximately, 4,000 acres of Shinnecock Bay were closed one month (10 April 2012) earlier than last year (6 May 2011) for one month's time (closure rescinded 11 May 2012). Our monitoring program also detected the presence of elevated *Alexandrium* densities in Sag Harbor Cove which led to the closure of this embayment on 26 April and was reopened one month later on 25 May 2012. In sum, more than 13,000 acres of shellfish beds were closed across Suffolk County due to *Alexandrium* blooms and PSP in 2012

During the spring of 2012, the first large-scale survey since the 1980s (Freudenthal and Jijina, 1988) was performed to assess the presence of DSP-producing *Dinophysis* in Long Island embayments (Fig. 10; Table 2). *Dinophysis* was observed at every site sampled (34 sites), and 21% of those sites had higher densities than those reported ~30 years ago (13,000 cells L^{-1}; Freudenthal and Jijina, 1988; Fig. 10; Table 2). *Dinophysis* densities at Britannia (site 2) and Woodbine (site 8) marinas, both sites located in Northport Harbor, reached 123,000 and 54,000 cells L^{-1}, respectively (Fig. 11). In 2012, the largest observed *Dinophysis* bloom occurred in Meetinghouse Creek (Fig. 12). This bloom lasted for ~2 months, reached >2 million cells L^{-1} and sustained densities over 10^4 cells L^{-1} for ~ 1 month (Fig. 12). Moreover, a smaller *Dinophysis* bloom (63,000 cells L-1) occurred in the adjacent embayment, Reeves Bay (Fig. 12). The 2012 Meetinghouse Creek bloom superseded the 2011 Northport Bay bloom which was 1.3 million cells L^{-1}.

DSP toxins in shellfish, 2010-2011- Both okadaic acid congeners (OA, DTX1) as well as pectenotoxins (PTX) were found in shellfish during the summer of 2010 and 2011 (Table 3, Fig. 13, 14), while DTX2 was not detected. During 2010, toxic shellfish were collected on 28-June, one day prior to the peak of the 2010 bloom, with site S4 having a higher toxin content (total OA congeners= 115 ng g^{-1}) than site S3 (total OA congeners= 52 ng g^{-1}) which was closer to the documented bloom (Fig. 1, 13, 14, Table 3). During 2011, OA, DTX1 and PTX levels in shellfish ranged from 24 - 818 ng g^{-1}, 13 - 455 ng g^{-1}, and 3 - 115 ng g^{-1}, respectively (Table 3, Fig. 13, 14), with the highest toxin concentrations (1245 ng g^{-1} total OA) found at site S3 (Woodbine Marina; Fig. 1) on 28-June. In 2011, five samples (four sites; S1, S2, S3 and S5) exceeded the USFDA action level (160 ng g^{-1} of shellfish tissue; black dotted line, Fig. 14, Table 3). While four of these samples were collected from areas already closed to shellfishing due to coliform bacteria, one of these samples was collected from an area open to shellfish harvest (S5, Fig. 1, 13, 14, Table 3). Esterified toxins represented 74 – 98 % of the total DSP toxins present in shellfish (Fig. 14). Prior to hydrolysis, only one shellfish sample from a region of Northport Harbor that was already closed to shellfish harvest (S3; 226 ng g^{-1}; Fig. 1, 13, 14) exceeded the USFDA action level. After hydrolysis, however, total DSP toxin concentrations increased by 4 – 63 fold (depending on shellfish species), thereby increasing the number of samples over the USFDA action level (one to five) and expanding to a region (S5; Fig. 1, 13, 14; Table 3) that was opened to harvest at the time of collection. This finding emphasizes the importance of analyzing for esterified toxins in order to properly manage shellfish beds in the state of NY.

***Objective 2*: Quantify the impact of anthropogenic stressors related to eutrophication including N and organic matter enrichment on the abundance of dinoflagellates and their toxins in LIS waters.**

Field sampling and analyses- To assess the impact of organic matter and nitrogen loading on *Alexandrium fundyense* and *Dinophysis acuminata* growth and their respective toxins a series of nutrient amendment experiments were performed during 2011 (26-April, 3-May, 9-May, 16-May, 6-June, 13-June, 21-June, 27-June and 6-July). Triplicate bottles (2.5 L) were filled with water from Northport Bay. An unamended control was established along with four treatments including 20 µM ammonium, 10 µM glutamine (=20 µM N), 100pM vitamin B_{12} and ~30 µM DON equivalent of high molecular weight organic matter from sewage treatment plant effluent (HMW STP). Similar experiments were conducted during 2012 (7-May, 15-May, 5-June, 19-June), with an unamended control and three treatments including 20 µM ammonium, ~30 µM DON equivalent of HMW STP and the addition of 20 µM ammonium + ~30 µM HMW STP. High molecular weight organic matter from sewage treatment plant effluent was isolated and concentrated from the Northport Sewage Treatment plant which is located in Northport Harbor. High molecular weight organic matter was isolated via tangential flow filtration as described by Gobler and Sañudo-Wilhelmy (2003). The use of tangential flow filtration ensures that high molecular weight organic material is concentrated but inorganic nutrient concentrations remained unchanged (Gobler and Sañudo-Wilhelmy, 2003). All treatment concentrations were chosen to match those which have previously elicited a growth response in *Alexandrium* cells (Leong et al., 2004) and were similar to peak elevated levels found in Long Island estuaries (Gobler et al., 2004). All bottles were incubated for ~ 48 h at ambient light and temperature at the Stony Brook Southampton Marine Science Center after which *A. fundyense* and *D. acuminata* cells were enumerated via the aforementioned methods. Differences among treatments were elucidated by means of a Two-Way ANOVA or with an appropriate non-parametric test when normality tests of log transformed data failed.

***Alexandrium* Nutrient Amendment Experiments-** In the spring of 2011, the additions of ammonium, and an organic source of N, glutamine, resulted in increased *Alexandrium* densities in 100% of the experiments conducted in Northport Bay with one (3-May) of those experiments having significantly ($p<0.001$, Student Newman Keuls) higher densities than those of the control (Fig 15). This suggests that both inorganic and organic forms of N can stimulate the growth of *Alexandrium*. Similarly, the addition of B_{12} and high molecular weight sewage treatment effluent increased *Alexandrium* densities in 100% of the experiments, while 50% (3-May, 9-May) of those increases were significantly higher than the control ($p<0.001$, Student Newman Keuls; Fig 15). In the spring of 2012, the additions of all three treatments (ammonium, HMW STP and ammonium + HMW STP) resulted in increased *Alexandrium* densities in an experiment conducted on 15 May (30 – 60% increases; Fig. 16). The addition of high molecular weight sewage treatment plant water (HMW STP) significantly increased *Alexandrium* densities ($p<0.05$, two-way ANOVA). This suggests that wastewater can promote *Alexandrium* blooms and even if you remove inorganic nitrogen from a sewage treatment system, organic matter may still promote the growth of *Alexandrium*. In addition, there was an antagonist interaction ($p<0.01$, two-way ANOVA) between the addition of ammonium and HMW STP water, whereby the addition of both decreased *Alexandrium* densities. In this case, the addition of ammonium may be suppressing transporters or the production of enzymes that target organic N, reducing the ability of these cells to use the HMW STP water.

***Dinophysis* Nutrient Amendment Experiments-** In the late spring to early summer of 2011 nutrient amendment experiments were conducted with Northport Bay water containing the DSP-producing dinoflagellate, *Dinophysis acuminata*, to assess the role of organic matter and inorganic N in promoting these blooms. When an inorganic N source, ammonium, and the vitamin, B_{12}, was added to *Dinophysis* bloom water *Dinophysis* densities significantly ($p<0.05$, Student Newman Keuls) increased compared to the control in 100% of the experiments conducted (Fig. 17). Similarly, the addition of glutamine significantly ($p<0.001$, Student Newman Keuls) increased (6-June, 13-June, 21-June) *Dinophysis* densities in 60% of the experiments conducted, while significantly ($p<0.05$, Student Newman Keuls) decreasing (27-June, 6-July) densities in 40% of the experiments conducted (Fig. 17). Similarly, the addition of HMW STP water increased *Dinophysis* densities in 80% of the experiments conducted with 75% of the increases (13-June, 21-June, 27-June) having significantly ($p<0.05$, Student Newman Keuls) higher densities compared to the control. In the late spring of 2012, the addition of all three treatments (ammonium, HMW STP and ammonium + HMW STP) increased *Dinophysis* densities (2 – 32%; Fig. 18), ammonium was the only significant ($p<0.001$, two-way ANOVA) treatment factor during an experiment conducted on 19 June. The sum of these results indicate that *Dinophysis* is directly or indirectly promoted by inorganic N loading and organic matter, perhaps more frequently than any other HAB on Long Island.

***<u>Objective 3</u>:* Quantify the impact of anthropogenic stressors related to climate change, including temperatures and CO_2, on the relative abundance of dinoflagellates and their toxins in LIS waters.**

CO_2 measurements in Northport Bay and LIS

Stationary deployment- To determine the CO_2 concentrations present during *Alexandrium* blooms, *in situ* measurements were made in the Northport Bay region. In 2011, CO_2 levels were measured during the *Alexandrium* bloom by the stationary deployment of a probe (HydroC™/CO_2, Contros), that makes continuous *in situ* measurements by way of infrared technology, at the primary site (2) in Northport Harbor. This instrument generates measurements of dissolved CO_2 *in situ* every 5 seconds and provides measurements of CO_2 in coastal systems consistent with the traditional measurements made on individual samples using standard methods and has been shown to be more accurate than other commercially available marine sensors (e.g. Sunburst) in coastal systems (ACT, 2010). To ground truth measurements made by the HydroC™/CO_2 probe deployed at site 2, total dissolved inorganic carbon (DIC) samples were collected from the same depth in the water column where the probe was deployed using a Van Dorn sampler. Water was transferred without bubbling to a 300 mL borosilicate bottle and samples were preserved using a saturated 1% mercuric chloride solution and kept at 4°C until analysis. pH measurements were made using an Oakton ® (± 0.01) calibrated prior to each use using NBS traceable standards. Measurements using this pH meter were never significantly different from scale corrected (Dickson 1993) spectrophotometric pH measurements made using *m*-cresol purple as described by Dickson et al. 2007. DIC samples were measured using an EGM-4 Environmental Gas Analyzer (PP Systems) system that quantifies total dissolved inorganic carbon levels (DIC) after separating the gas phase from seawater using a Liqui-Cel Membrane (Membrana; Talmage and Gobler 2009). This instrument generally provides a methodological precision better than ± 5% for replicated measurements of total dissolved inorganic carbon and has provided full recovery (>100%) of Dr. Andrew Dickson's (University of California San Diego, Scripps Institution of Oceanography) certified reference material (Batch 102 and 123). Total dissolved inorganic carbon and pH of the Dickson standard was quantified with each analytical run as a quality assurance measure. CO_2 levels were calculated using measured levels of DIC, pH (NBS scale), temperature, and salinity, as well as the first and second dissociation constants of carbonic acid in seawater according to Roy et al. (1993) using the program CO2SYS (http://cdiac.ornl.gov/ftp/co2sys/).

Horizontal transect- In addition to the CO_2 measurements made via stationary deployment, the spatial variability in pCO_2, chlorophyll *a*, and salinity during blooms was assessed in May 2012 by conducting a transect from Northport Harbor to Northport Bay. A similar cruise was conducted where just pCO_2 was assessed in vertical profiles at locations from the western portion of Long Island Sound towards the east (ending in Port Jefferson). The HydroC™/CO_2 probe and a YSI 6920v2 (YSI Inc., Yellow Springs, OH) were attached side-by-side to a stabilizing bracket that was mounted on the side (towards the stern) of a small vessel so that probes were at a depth of 0.5m. During the horizontal transect the vessel moved well below wake speed to minimize turbulent mixing around the probes. Additionally, the time signatures of both probes were linked to a GeoChron Blue GPS tracking and data logger to track their measurements through space and time. Heat maps of these parameters were created using the geostatistical analyst extension in ARCGIS 10 using standard kriging methods.

Temperature and CO_2 experiments- To assess the effects of temperature on the growth and toxicity of *Alexandrium fundyense*, a series of temperature manipulation experiments were conducted. Triplicate bottles (2.5 L) were filled with water from Northport Bay, 20µM ammonium and 2µM P were added to each bottle and bottles were incubated at two different temperatures (15ºC, 19ºC) for 48 h. Additionally, to assess the effects of different CO_2 levels on the growth and toxin production of the PSP-producing dinoflagellate, *Alexandrium fundyense,* Northport Bay water was incubated at ambient light and temperature under three different levels of CO_2 (390 (ambient), 750, 1500 µatm). A gas proportionator system (Cole Parmer® Flowmeter system, multitube frame) was used to deliver ambient air (390µatm), and premixed CO_2 gas (750 and 1500 µatm; Praxair) to seawater treatments at a net flow rate of 300 ± 5 mL min^{-1} which were continuously delivered to the bottom of triplicate, polycarbonate, 2.5-L bottles (Rose et al., 2009) using airstones. This delivery rate will turn over the volume experimental bottles >100 times daily, ensuring proper CO_2 concentrations were maintained (Talmage and Gobler, 2010). Bottles were filled with 50% Northport Bay water and 50% 0.2micron filtered Northport Bay water. Additional experiments were conducted to assess the effects of varying levels of CO_2 on the growth of phytoplankton communities from Long Island Sound. Bottles containing phytoplankton communities from both the western and eastern ends of Long Island Sound were exposed to ambient CO_2 levels and 1500µatm as above. CO_2 levels achieved within experimental bottles were confirmed via direct measurements using an EGM-4 Environmental Gas Analyzer (PP Systems) system that quantifies total dissolved inorganic carbon levels (TDIC) after separating the gas phase from seawater using a Liqui-Cel Membrane (Membrana). CO_2 levels were then subsequently calculated using measured levels of TDIC, pH (NBS scale), temperature, and salinity for each experiment, as well as the first and second dissociation constants of carbonic acid in seawater according to Roy et al. (1993) using the program CO2SYS (http://cdiac.ornl.gov/ftp/co2sys/). Multiple pH measurements were made throughout each experiment using a hand-held Orion 3-star plus which was calibrated prior to each use using NIST traceable standards 4.01, 7 and 10.01 (Thermo Scientific). Bottles were amended with nutrients (dilutions of *f*/2 stock media with N:Si ratio of 1:1) and both batch and semi-continuous (a known amount of water was removed during the midpoint of the experiment and that same amount of fresh 0.2micron filtered water was added back into to experimental bottles) methodologies were used. All bottles were incubated for 3-6 days at ambient light and temperature at the Stony Brook Southampton Marine Science Center after which *A. fundyense* cells and their respective toxins were quantified via the aforementioned methods. Differences among treatments were elucidated by means of a One-Way ANOVA with multiple comparison tests (i.e. Student-Newman-Keuls) or with an appropriate non-parametric test when normality tests of log transformed data failed.

2011 temporal CO_2 and *Alexandrium* bloom dynamics- During spring 2011, *Alexandrium* densities were present from late March through late May, with the largest peak occurring on 9 May at 25,300 cells L^{-1} and a smaller secondary peak on 16 May reaching 6,600 cells L^{-1} (Fig 19). Total phytoplankton biomass was significantly lower during the peak of the *Alexandrium* bloom (3- 24 May; 3.3 ± 0.9 µg chlorophyll *a* L^{-1}; Mann-Whitney Rank Sum test, $p<0.01$) compared to before (28 March –29 April) and after (1- 6 June) the bloom (11.5 ± 2.1 µg chlorophyll *a* L^{-1}; Fig. 19). During the *Alexandrium* bloom, a probe (HydroC™/CO_2; Contros) deployed on 5 May in Northport Harbor recorded pCO_2 concentrations ranging from 235µatm (7 May) to 1799µatm (21 May; Fig. 19). The first peak of the *Alexandrium* bloom coincided with lower CO_2 levels (9 May; 350 – 560µatm), while the secondary peak (16 May) occurred during elevated CO_2 levels (590 – 1000µatm; Fig. 19). CO_2 levels measured from discrete DIC samples were inversely correlated with total chlorophyll *a* concentrations (R= -0.77). While pCO_2 levels fluctuated daily, overall levels as well as the range of probe measured values increased over the length of the deployment (Fig. 19). Additionally, while pCO_2 levels measured by the probe were always lower (40 to 220 µatm; 3 - 22%) compared to the discrete DIC samples collected to ground truth the probe, levels of CO_2 measured using both of these methodologies were highly correlated (R^2=0.92). Our results are consistent with past research investigating the allelopathic interactions between *Alexandrium* and other phytoplankton (Hattenrath-Lehmann and Gobler, 2011) with chlorophyll *a* concentrations decreasing as *Alexandrium* densities increase. Progressively increasing pCO_2 concentrations over the course of the bloom are suggestive that *Alexandrium* may influence the pCO_2 of the surrounding environment; potentially via secreting allelochemicals that are known to cause the lysis or growth inhibition of competing phytoplankton. This interaction has the potential to affect pCO_2 concentrations by: 1) lysed phytoplankton exuding organics that would be respired by microbes which would ultimately increase bacterial levels and increase pCO_2, and 2) by decreasing overall phytoplankton concentrations (as evidenced by decreased chlorophyll) and therefore decreasing pCO_2 uptake; both scenarios would act to synergistically increase pCO_2 concentrations. This theory is further substantiated by the increase in chlorophyll *a* and concurrent drawdown of CO_2 after the demise of the bloom. Other sources of pCO_2 in this region may include groundwater input or the near-by sewage treatment plant. It is also possible that increasing temperatures fostered increasing rates of benthic and/or pelagic microbial respiration and CO_2 production. While we cannot constrain the precise mechanism, our data clearly demonstrates that the *Alexandrium* bloom in Northport Bay during 2011 coincided with elevated and rising levels of pCO_2.

2012 Spatial pCO_2 and *Alexandrium* cell distribution in Northport Bay - On 16 May 2012, a cruise was conducted to assess a variety of water quality parameters including the spatial distribution of *Alexandrium* densities, pCO_2 concentrations, salinity, and chlorophyll *a* concentrations in the Northport Bay region (Fig. 20). *Alexandrium* densities ranged from 180 – 8,300 cells L^{-1} with the highest densities occurring in Northport Harbor (site 2) and gradually decreasing towards Northport Bay (site 10; Fig. 20A). Similarly, using the HydroC™/CO_2 probe, a transect conducted from Northport Harbor into Northport Bay (and back) measured CO_2 concentrations that ranged from 360 – 1230µatm. The highest levels (>1000µatm) of pCO_2 were confined to the Northport Harbor region and decreased towards the bay (<500µatm) with additional high CO_2 water intrusions (~800µatm) north of the bay where another enclosed harbor region exchanges with the bay (Fig. 20B). Contrastingly, salinity was lower in Northport Harbor (< 24psu) and increased (~26 psu) towards the bay, with evidence of additional freshwater input in the eastern portion of the bay where salinity decreased slightly (Fig. 20C). Chlorophyll *a* concentrations ranged from 1- 19 µg L^{-1} with lower values measured in the Harbor (<9 µg L^{-1}) and increasing concentrations towards the bay (Fig. 20D). The *Alexandrium* and chlorophyll *a* dynamics were consistent with our stationary deployment of the pCO_2 probe in 2011: high *Alexandrium* densities were associated with low chlorophyll *a* concentrations. Similar to our stationary deployment, high pCO_2 levels were associated with low chlorophyll *a* concentrations. Exceptionally high levels of pCO_2 in the back portion of Northport Harbor compared to the Bay could also be due to the influx of groundwater and sewage treatment plant water (Fig. 20A) which would potentially have high pCO_2 levels due to high bacterial levels and respiration rates in these waters. The influence of these freshwater inputs are shown by the lower salinity measured in the Northport Harbor region (Fig. 20C). It is also possible that organically enriched sediment in this region fostered higher rates of benthic microbial respiration and CO_2 production. Overall the increased *Alexandrium* densities and pCO_2 concentrations in Northport Harbor as well as the sharp salinity gradient between the Bay and the Harbor are indicative of a long residence time in the Harbor region which may promote these blooms via positive feedback to the system: Decreased flushing rates would retain nutrients and organic matter, increase bacterial loads/respiration, increase the organic loads to the sediments all of which would enhance CO_2 levels in the Harbor and overall make Northport Harbor a net heterotrophic system.

***Alexandrium* temperature manipulation experiments and *Alexandrium* densities under varying CO_2 levels-** In 50% (2 of 4) of the temperature manipulation experiments conducted during 2012, *Alexandrium* densities were significantly higher (60-100%; $p<0.01$, t-test) when incubated at 15ºC compared to 19ºC (24 April, 30 April; Fig. 21). The other 50% of these experiments resulted in *Alexandrium* densities increasing up to 38% (15 May) when incubated at 19ºC compared to 15ºC; however, these increases were not significant. Significant increases in *Alexandrium* densities when incubated at 15ºC are consistent with past research demonstrating that field populations grow maximally at temperatures close to 15ºC (Hattenrath et al., 2010). Notably, the change in results as bay waters warmed suggests that there was a shift in the clonal composition of the bloom toward more heat tolerant strains over the course of the bloom or that the population was well acclimated to the ambient temperatures present over the course of the bloom.

In an experiment conducted on 9 May 2011, *Alexandrium* densities significantly increased under increasing CO_2 levels (Fig. 22), both 750µatm (~83,216 cells L^{-1}; $p<0.01$, Student Newman Keuls) and 1500µatm (~96,750 cells L^{-1}; $p<0.001$, Student Newman Keuls), compared to that of ambient (390µatm) CO_2 levels (~75,936 cells L^{-1}; Fig. 22). These values are a 10% and 27% increase in *Alexandrium* densities compared to ambient CO2 levels for 750µatm and 1500µatm, respectively (Fig. 22). New toxin data demonstrates that while GTX5 and C2 toxin congeners as well as total toxicity per cell increases under increasing CO_2 levels, however, these increases are not statistically significant (Fig. 22). These experiments demonstrate that under increasing CO_2 levels, which are due to either climate change or anthropogenic nutrient loading, *Alexandrium* blooms may intensify.

pCO_2 levels and effects of varying CO_2 levels on Long Island Sound's phytoplankton communities- During early August surface pCO_2 concentrations approached 700µatm, with the highest concentrations occurring in the western portion of the Sound (Fig. 23). Concentrations increased during late August surpassing 750µatm with higher concentrations expanding towards the eastern part of the Sound (Fig. 23). Experiments conducted using both Western and Eastern Long Island Sound water demonstrated that in both areas diatom and autotrophic nanoflagellate densities were higher than dinoflagellate densities (Fig. 24). In the Western Sound increasing CO_2 levels (1500µatm) significantly ($p<0.05$) decreased total dinoflagellate densities (including *Prorocentrum* sp.) and densities of the diatom *Cylindrotheca* sp. compared to the control (390µatm: Fig. 24). In the Eastern Sound increasing CO_2 levels (1500µatm) significantly ($p<0.05$) increased total dinoflagellate densities and autotrophic nanoflagellates while decreasing densities of the diatom *Cylindrotheca* sp. compared to the control (390µatm: Fig. 24). Overall, our results suggest that phytoplankton species are differentially affected by changing CO_2 levels and that more research is needed to fully understand the effects of these stressors on phytoplankton communities.

C2. Scientific Abstract:

This study investigated the distribution and causes of the PSP-producing and DSP-producing dinoflagellates, *Alexandrium fundyense*, and *Dinophysis acuminata*. During the study, both *Alexandrium* and *Dinophysis* were present at >30 sites across Long Island. The largest *Alexandrium* blooms (>10^4 cells L^{-1}) were observed in Northport Bay, Mattituck Inlet, Weesuck Creek (Shinnecock Bay) and Meetinghouse Creek some of which were closed to shellfish harvest due to the presence of saxitoxin contaminated shellfish that were over the federal closure limit of 80µg STX eq./100g of shellfish. Since 2005, PSP-induced shellfish bed closures in NY have expanded from 0 to >13,000 acres of shellfish beds closed in 2012; these closures may continue to expand in the future. The largest *Dinophysis* blooms (>10^4 cells L^{-1}) occurred in Northport Bay, Meetinghouse Creek and Reeves Bay. The 2012 Meetinghouse Creek *Dinophysis* bloom was the largest recorded anywhere, lasting for ~2 months, reaching >2 million cells L^{-1} and sustaining densities over 10^4 cells L^{-1} for ~ 1 month. For *Dinophysis*, PTX concentrations were usually the most abundant particulate toxin followed by esterified OA, esterified DTX1, free DTX1 and free OA, while no DTX2 was observed. While DSP contaminated shellfish approaching 1300 ng/g were collected from Northport Bay in 2011 and were over the federal closure limit (160 ng/g of shellfish tissue), no closures were implemented. Experiments suggested that both *Alexandrium* and *Dinophysis* blooms are enhanced by different types of nutrients that contain nitrogen, phosphorus, and organic compounds. For *Dinophysis*, these effects of inorganic and organic N on cell densities may be direct or indirect. However, this alga was promoted by N loading more consistently than *Alexandrium* and most other HABs in NY. Additionally, experiments conducted to assess future climate change scenarios suggest that *Alexandrium* thrive in a certain temperature (15°C) window and that *Alexandrium* densities and toxicity may be enhanced by increasing CO_2 levels. Moreover, our research suggests that Long Island estuaries have already surpassed future climate change projections (>750µatm by 2100) due to eutrophication-enhanced acidification. As such, eutrophication-driven CO_2 enrichment must be considered as a factor that may promote *Alexandrium* blooms in NY.

C.

C3. Problems Encountered: Originally, the methodology used to analyze our shellfish samples only included free toxins, however the FDA recognizes both free and esterified toxins in DSP shellfish closure limits. Therefore we needed to change our procedure to include sample hydrolysis which allows for the detection of esterified toxins. Upon hydrolyzing samples we found a significant amount of esterified toxins present in our New York samples thus increasing the total DSP toxin concentrations in all samples. This important finding lead to the discovery of shellfish samples over the FDA recommended closure limit.

C4. New Research Directions: One of the original objectives of this project was to quantify the impact of anthropogenic stressors related to climate change, including CO_2, on the relative abundance of toxic dinoflagellates and their toxins in LIS waters. The intention was to execute this objective solely using an experimental based methodology (i.e. by conducting CO_2 addition experiments as above). However, to determine if this was a locally relevant question we included field work that was designed to assess the spatial and temporal dynamics of CO_2 concentrations in the Northport Bay region as well as Long Island Sound. This field work was conducted using a probe (Hydro C™/CO_2, Contros) that was deployed at a fixed point in Northport Bay and was also attached to a boat to conduct horizontal transects throughout Northport bay and LIS. We believe that this addition significantly enhanced both the research and our understanding of the Northport region and LIS. Research conducted during this NYSG funded project aided in obtaining MERHAB funding for continuing research related to these toxin-producing dinoflagellates.

C5. Interactions: We have been in constant contact with personnel from multiple agencies regarding shellfish toxicity in NY, including: NYSDEC (Karen Chytalo, Karen Graulich, Bill Hastback), NOAA's Marine Biotoxin Laboratory (Steve Morton) as well as the FDA (Jonathan Deeds).

C6. Presentations and Publications

Publications:

Hattenrath-Lehmann, T.K., and C. J. Gobler. 2011. Allelopathic inhibition of competing phytoplankton by North American strains of the toxic dinoflagellate, *Alexandrium fundyense*: evidence from field experiments, laboratory experiments, and bloom events. *Harmful Algae*. 11: 106-116.

Anglès, S., E. Garcés, T. K. Hattenrath-Lehmann, and C. J. Gobler. 2012. In situ life-cycle stages of *Alexandrium fundyense* complex during a bloom development in New York (USA). *Harmful Algae*. 16: 20-26.

Hattenrath-Lehmann, T.K., M. A. Marcoval, D. L. Berry, S. Fire, Z. Wang, S. L. Morton, and C. J. Gobler. 2013. The emergence of *Dinophysis acuminata* blooms and DSP toxins in shellfish in New York water. *Harmful Algae*. 26: 33–44

Hattenrath-Lehmann, T.K., Wallace R.B., Koch F., Mittelsdorf H. Goleski J.A., Smith, J.L., Anderson, D.A. Gobler, C. J. In prep. The effects of elevated CO_2 on the growth and toxicity of field populations and cultures of the PSP-producing dinoflagellate, *Alexandrium fundyense*. Limnology and Oceanography

Presentations:

Gobler C.J., and T.K. Hattenrath-Lehmann. 2011. Factors promoting blooms of the PSP- and DSP-producing dinoflagellates, *Alexandrium fundyense* and *Dinophysis acuminata*, in Long Island Sound. The Northeast Estuarine Research Symposium. Port Jefferson, NY. May 2011. Oral Presentation.

Hattenrath-Lehmann, T.K., S.L. Morton, and C.J. Gobler. 2011. A tale of two dinoflagellates: Co-occurring blooms of the PSP- and DSP-producing dinoflagellates, *Alexandrium fundyense* and *Dinophysis acuminata*, in a New York estuary. 6th Symposium on Harmful Algae in the US. Austin, TX. November 2011. Oral Presentation.

Hattenrath-Lehmann, T.K., and C.J. Gobler. 2012. The PSP- and DSP-producing dinoflagellates, *Alexandrium fundyense* and *Dinophysis acuminata*, and shellfish toxicity in New York estuaries. Stony Brook Southampton Coastal & Estuarine Research Program Environmental Symposium. Southampton, NY. April 2012. Poster Presentation.

Hattenrath-Lehmann, T., S.L. Morton, and C.J. Gobler. 2012. The emergence of toxic *Dinophysis acuminata* blooms in a New York estuary. 15th International Conference on Harmful Algae. Changwon, Korea. October 2012. Poster Presentation. Maureen Keller Award, Best Student Poster.

Gobler, C.J., T.K. Hattenrath-Lehmann, Y.Z. Tang, and F. Koch. 2012. Tragedy of the commons: Eutrophication, acidification, and the expansion of HABs across Long Island, NY, USA. 15th International Conference on Harmful Algae. Changwon, Korea. October 2012. Oral Presentation.

Hattenrath-Lehmann, T.K., J.A. Goleski, H. Mittelsdorf, and C.J. Gobler. 2013. The expansion of the PSP- and DSP-producing dinoflagellates, *Alexandrium fundyense* and *Dinophysis acuminata*, and shellfish toxicity across Long Island. Stony Brook Southampton Coastal & Estuarine Research Program Environmental Symposium. Southampton, NY. April 2013. Poster Presentation.

Gobler C.J., and T.K. Hattenrath-Lehmann. 2013. Continued expansion of *Alexandrium* blooms and PSP across Long Island Sound. Long Island Sound Research Symposium. Port Jefferson, NY. April 2013. Oral Presentation.

D. Accomplishments:

D1. Impacts & Effects: Shellfish toxicity data from our study clearly show that the DSP-producing dinoflagellate, *Dinophysis acuminata*, is a real threat to our embayments here in New York as some areas open to shellfishing should have been closed due to toxin levels exceeding the FDA closure limit. The National Shellfish Sanitation Program currently provides no guidance for appropriate testing methods for DSP, which would make it difficult for environmental managers to close and then re-open shellfish beds that tested positive for DSP toxins. On a positive note, we alerted the FDA of our DSP issue in NY and steps have been taken to submit a multi-lab validation effort to the ISSC (Interstate Shellfish Sanitation Conference) using shellfish collected from our project along with those collected from other DSP problem areas such as Washington and Texas. This multi-lab effort seeks to gain approval from the ISSC for use of either the LC/MS/MS or the more affordable Abraxis PP2A kit to measure DSP toxins in shellfish so that state agencies such as the NYDEC can properly manage shellfish beds and protect public health.

D2. Scholar(s) & Student(s) Status: NYSG Scholar, Theresa Hattenrath-Lehmann completed her departmental exams in the fall of 2009 and defended her proposal in August 2012. Her anticipated graduation date is May 2014.

D3. Volunteers: Many Gobler lab members who were not supported by this project have assisted in field sampling and laboratory sample processing for this project. They include Ryan Wallace, Jennifer Goleski, Heidi Mittelsdorf, Florian Koch, Alejandra Marcoval, Lucas Merlo, and Matthew Harke. In addition, an undergraduate student volunteer, Gene Oh, assisted with the enumeration of *Dinophysis* cells.

D4. Patents: No patents pending.

E. Stakeholder Summary:

This study investigated the distribution and causes of the PSP-producing and DSP-producing dinoflagellates, *Alexandrium fundyense*, and *Dinophysis acuminata*. During the study, both *Alexandrium* and *Dinophysis* were present at over 30 sites across Long Island. The largest *Alexandrium* blooms were observed in Northport Bay, Mattituck Inlet, Weesuck Creek (Shinnecock Bay) and Meetinghouse Creek some of which were closed to shellfish harvest due to the presence of saxitoxin contaminated shellfish. Since 2005, PSP-induced shellfish bed closures have expanded from 0 to >13,000 acres of shellfish beds closed in 2012; these closures may continue to expand in the future. The largest *Dinophysis* blooms occurred in Northport Bay, Meetinghouse Creek and Reeves Bay. While DSP contaminated shellfish were collected from Northport Bay and were over the federal closure limit, no closures were implemented. Experiments suggested that both *Alexandrium* and *Dinophysis* blooms are caused by different types of nutrients that contain nitrogen and organic compounds. Additionally, *Alexandrium* densities and toxicity may be enhanced by increasing CO_2 levels common in eutrophic estuaries.

F. Pictorial:.

The HydroC/CO_2 probe (Contros). Photo taken by Theresa Hattenrath-Lehmann.

The Hydro C/CO_2 probe (Contros) attached to a boat for taking spatial measurement of CO_2 levels in Northport Bay during May 2012. Photo taken by Theresa Hattenrath-Lehmann.

FIGURES:

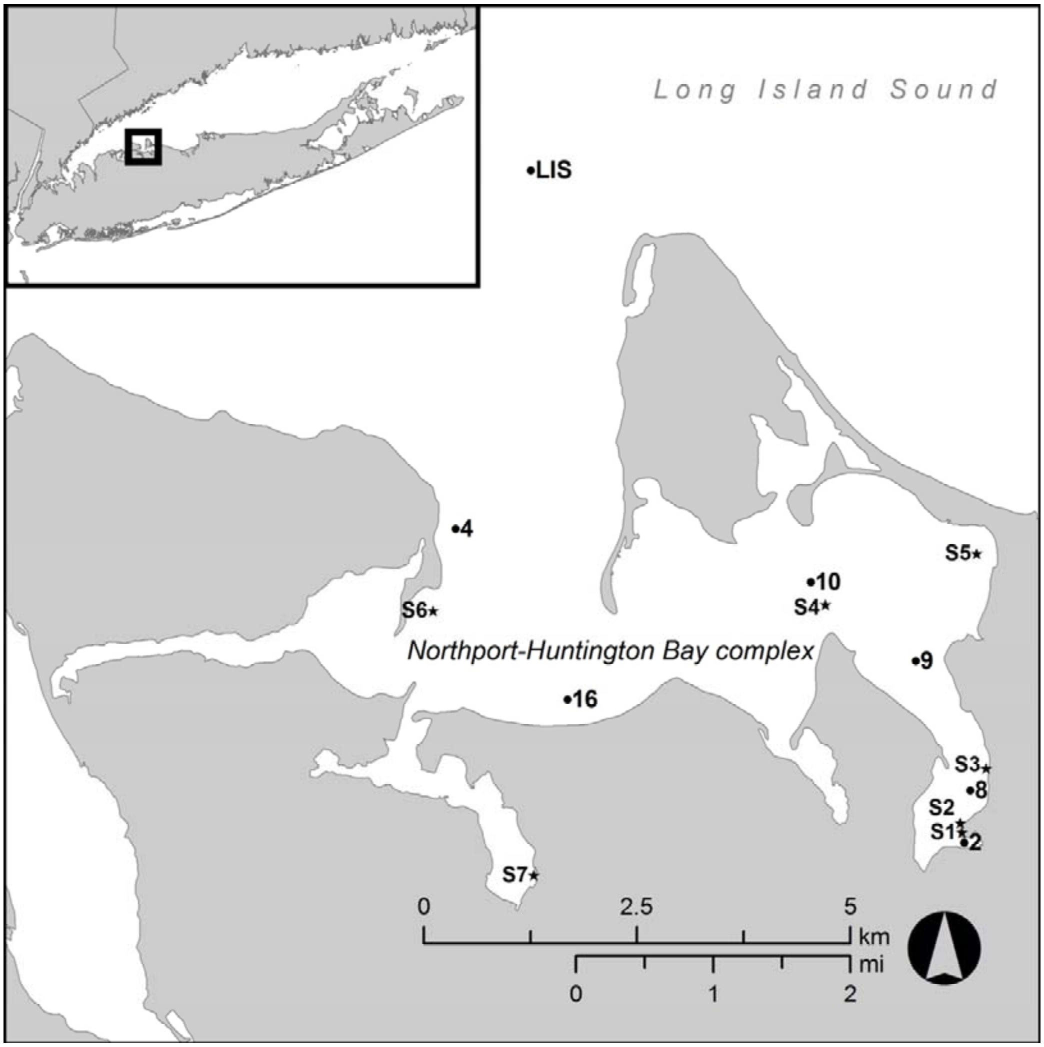


Figure 1- Field sampling (black circles) and shellfish collection (black stars) locations in Northport-Huntington Bay complex, New York.

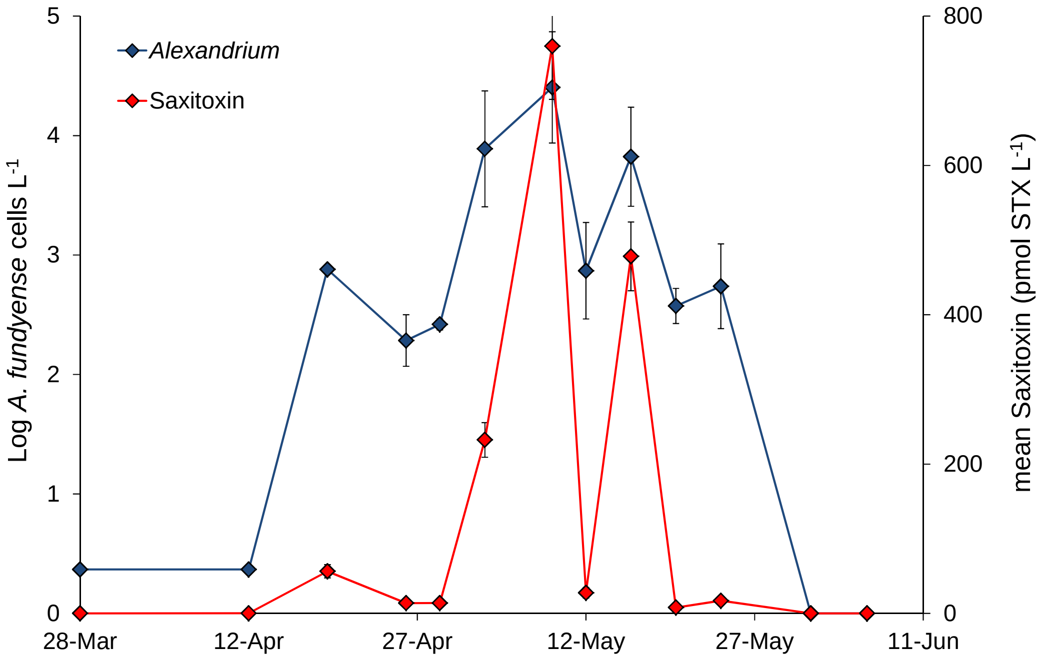


Figure 2. Log *Alexandrium* densities cells L^{-1} and saxitoxin concentrations (pmol STX L^{-1}) in Northport Harbor, NY during spring 2011.

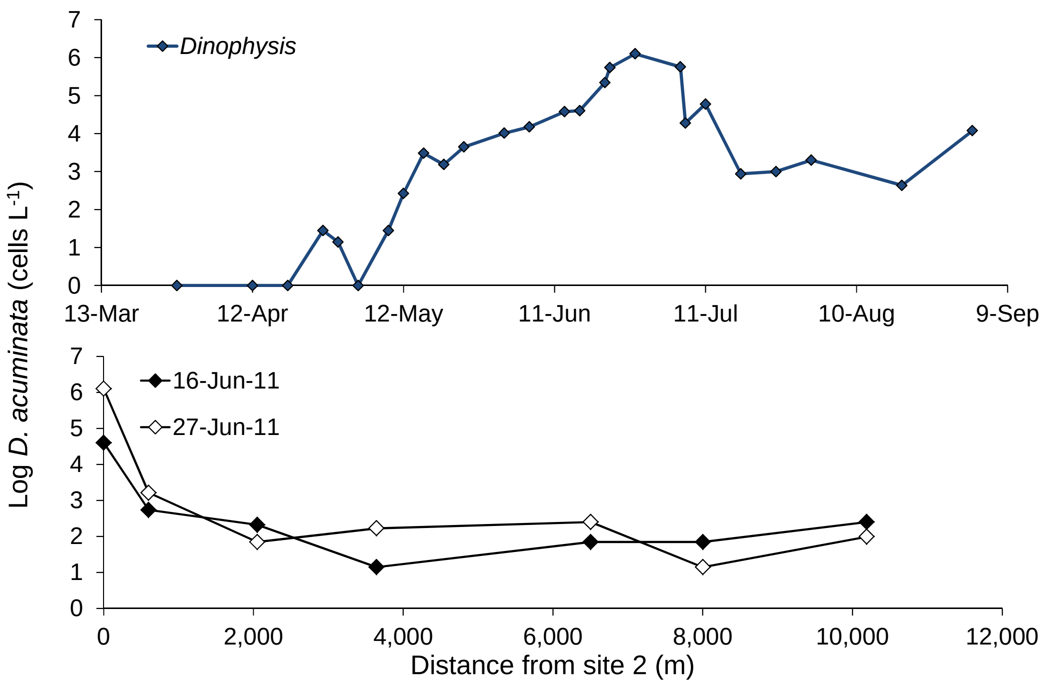


Figure 3. Top panel: Log *Dinophysis acuminata* densities (cells L^{-1}) in Northport Harbor, NY during spring 2011; Bottom panel: Log *Dinophysis acuminata* densities (cells L^{-1}) for cruises conducted in Northport Bay, New York, during 16 June and 27 June 2011 as a function of distance from site 2.

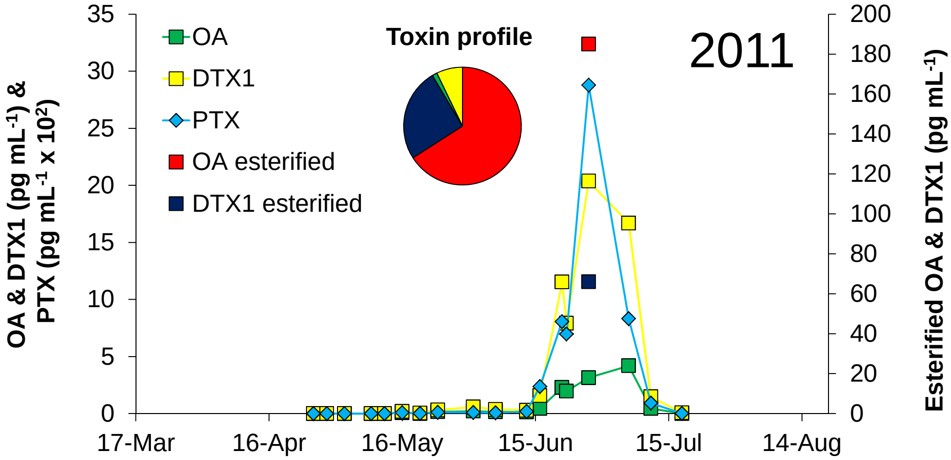


Figure 4. DSP toxins (free and esterified okadaic acid (OA) and dinophysistoxin1 (DTX1)) and associated pectenotoxins (PTX) from phytoplankton concentrates collected during the extraordinary 2011 *Dinophysis* bloom (1.3 x 10^6 cells L^{-1}). Inset: Toxin profile of hydrolyzed phytoplankton concentrates expressed as the mean of each toxins contribution to the total toxin profile.

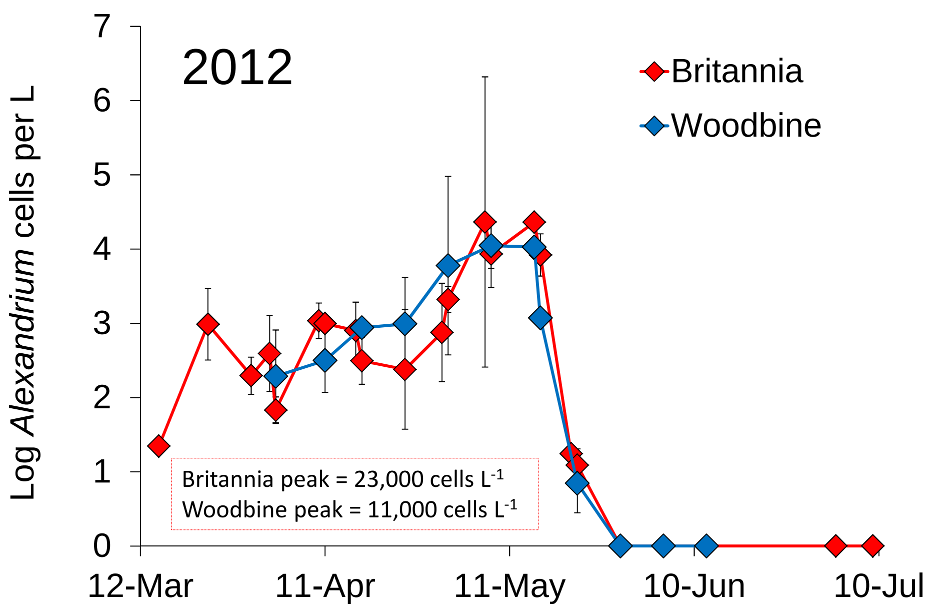


Figure 5. Log *Alexandrium* densities in cells L^{-1} for Britannia (site 2) and Woodbine (site 8) both located in Northport Harbor, NY during spring 2012.

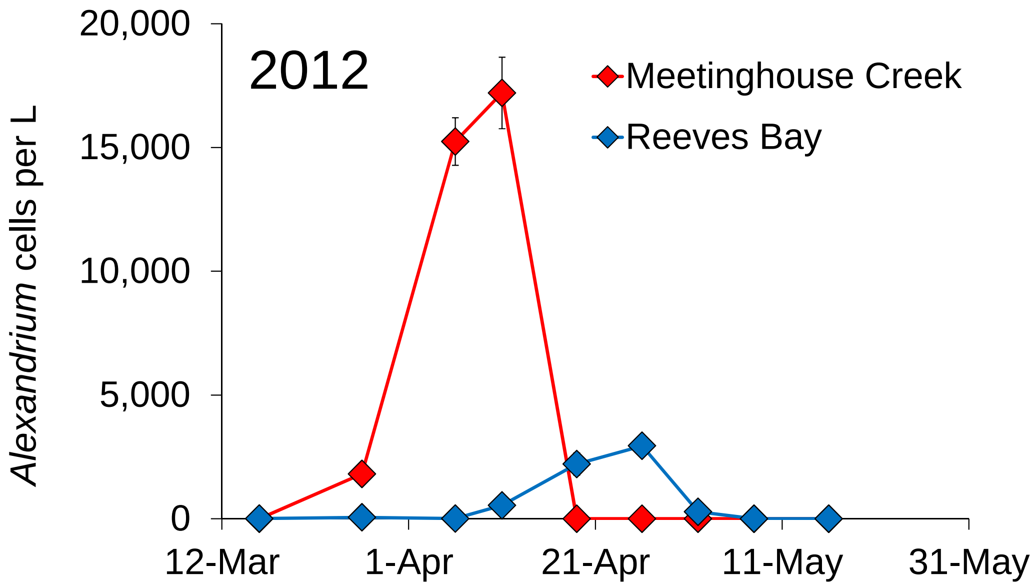


Figure 6. *Alexandrium* densities in cells L^{-1} for East End Long Island Sites, Meetinghouse Creek and Reeves Bay, during spring 2012.

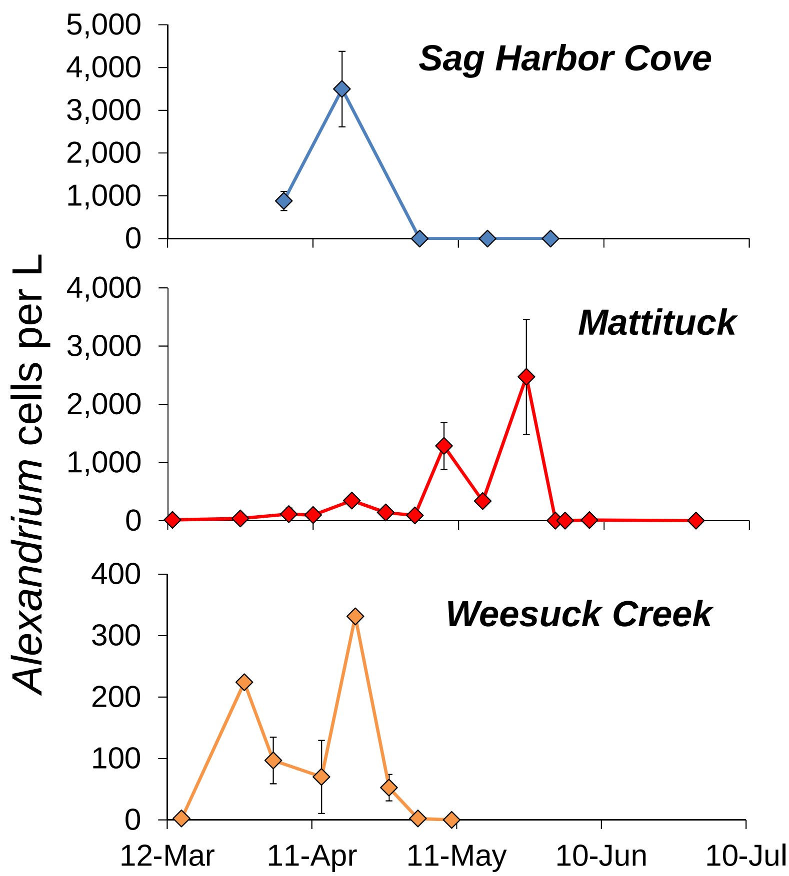


Figure 7. *Alexandrium* densities in cells L^{-1} for south shore (Weesuck Creek) and east end (Sag Harbor Cove and Mattituck) Long Island, NY shellfish bed closure sites during spring 2012.

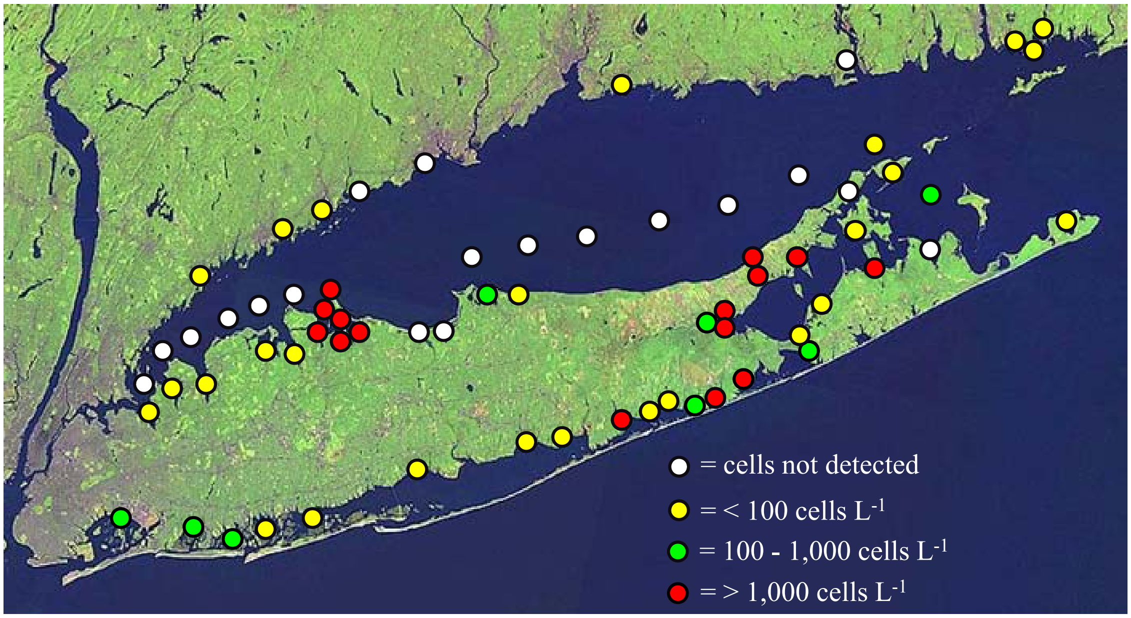


Figure 8. The distribution of PSP-producing *Alexandrium* along Long Island, NY and CT. Circles indicate the highest observed densities of *Alexandrium* (cells L^{-1}) found at each site during 2007 - 2012.

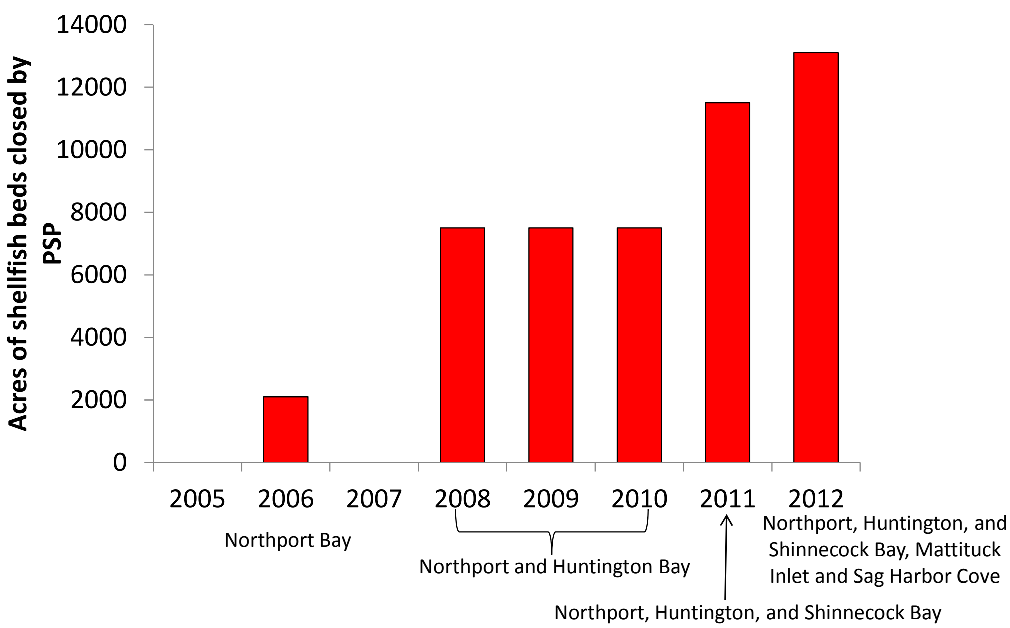


Figure 9. The expansion of PSP-induced shellfish bed closures on Long Island, 2005 – 2012. Prior to 2006, Long Island had never experienced a PSP event.

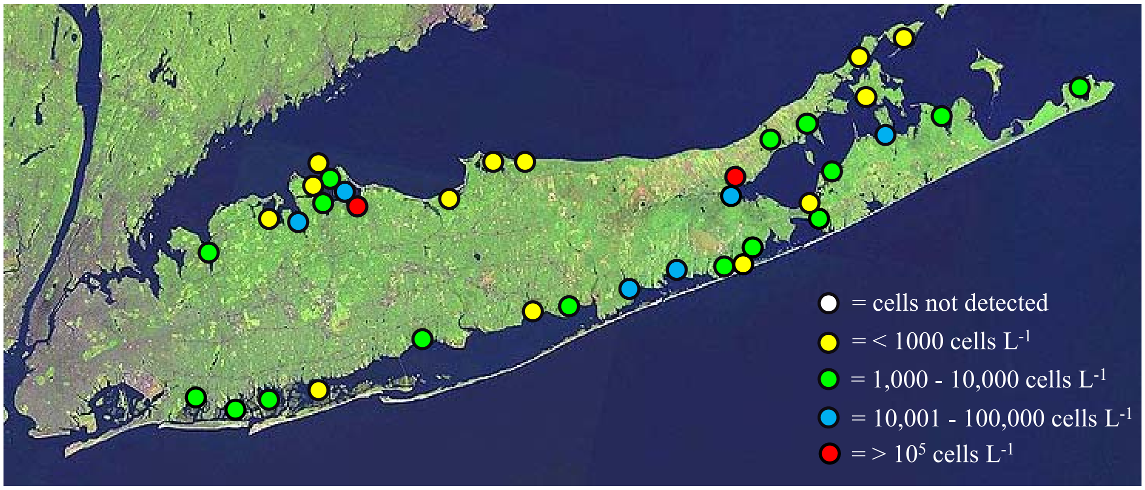


Figure 10. The distribution of DSP-producing *Dinophysis* along Long Island. Circles indicate the highest observed densities of *Dinophysis* (cells L^{-1}) found at each site during 2008 - 2012.

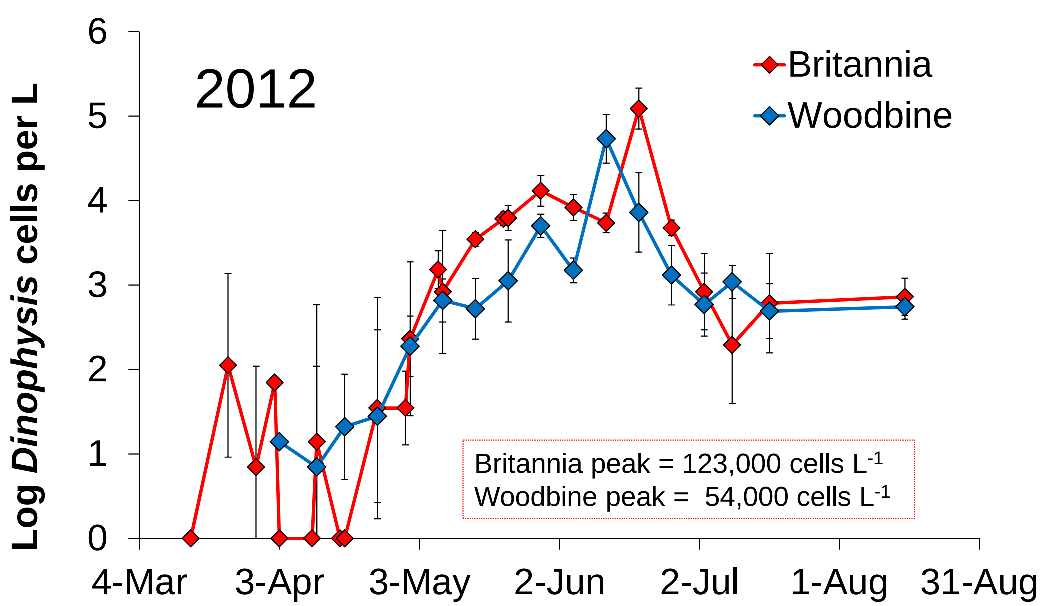


Figure 11. Log *Dinophysis* densities in cells L^{-1} for Britannia (site 2) and Woodbine (site 8) both located in Northport Harbor, NY during spring 2012.

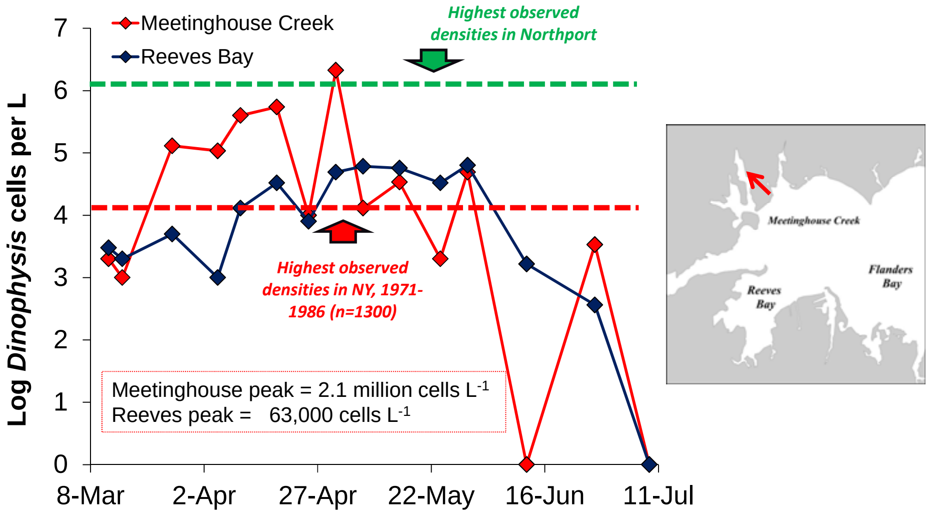


Figure 12. *Dinophysis* densities in cells L^{-1} for East End Long Island Sites, Meetinghouse Creek and Reeves Bay, during spring 2012.

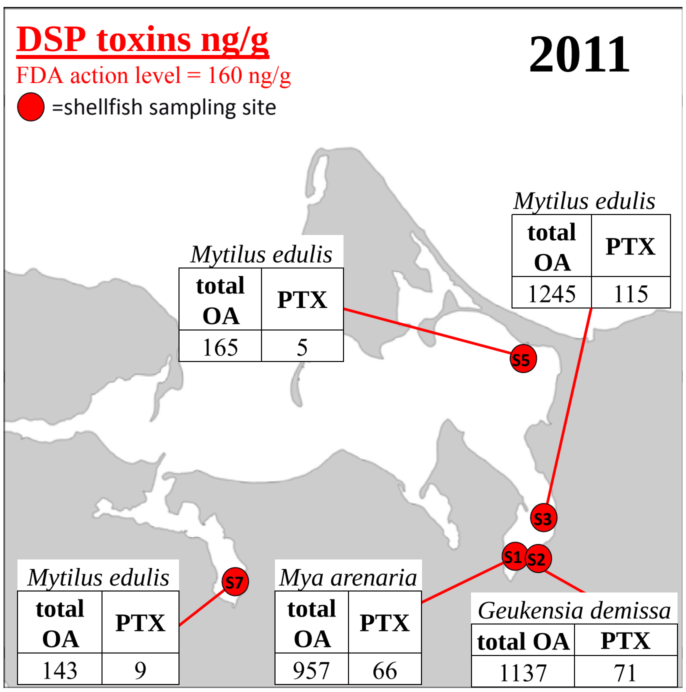


Figure 13. Total DSP toxins (OA + DTX1) & pectenotoxins (PTX) in wild (*Mya arenaria* & *Geukensia demissa*) & indicator shellfish species (*Mytilus edulis*) collected from Northport Harbor during late June to early July of 2011. The FDA action level for DSP is 160 ng/g of shellfish tissue & includes both free & esterified okadaic acid congeners (OA + DTX1).

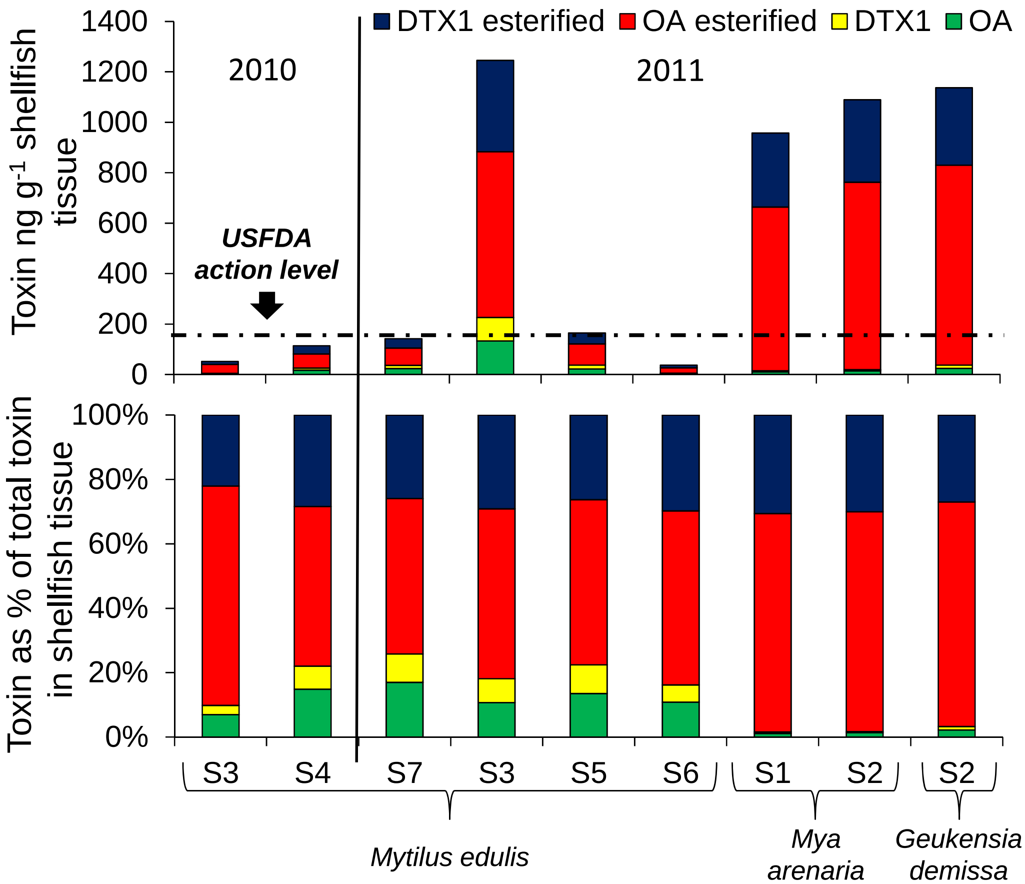


Figure 14. Top panel: Okadaic acid (OA), dinophysistoxin 1 (DTX1) & their esters (ng g^{-1}) measured in shellfish from the Northport-Huntington Bay complex located in New York, USA during 2010 & 2011. The USFDA action level (160 ng g^{-1} of shellfish tissue) is indicated by the black dotted line. Bottom panel: Okadaic acid (OA), dinophysistoxin 1 (DTX1) & their esters as a percentage of total DSP toxins in shellfish tissue. Sites S1 - S7 as in Table 3.

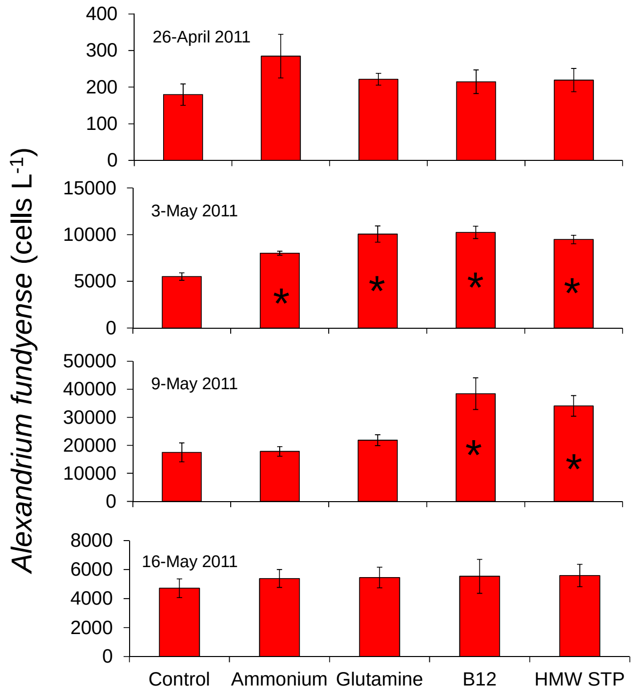


Figure 15. *Alexandrium fundyense* densities (cells L^{-1}) following nutrient amendment experiments conducted with Northport Bay water in the spring of 2011. Bars are means while error bars represent SD of triplicate measurements. Asterisks denote treatments significantly different from the control.

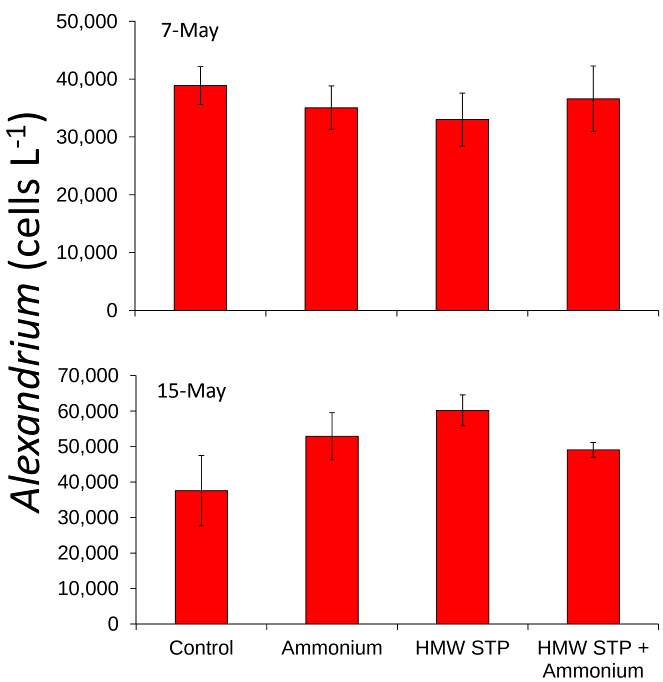


Figure 16. *Alexandrium* densities (cells L^{-1}) following nutrient amendment experiments conducted with Northport Bay water in the spring of 2012. Bars are means while error bars represent SD of triplicate measurements.

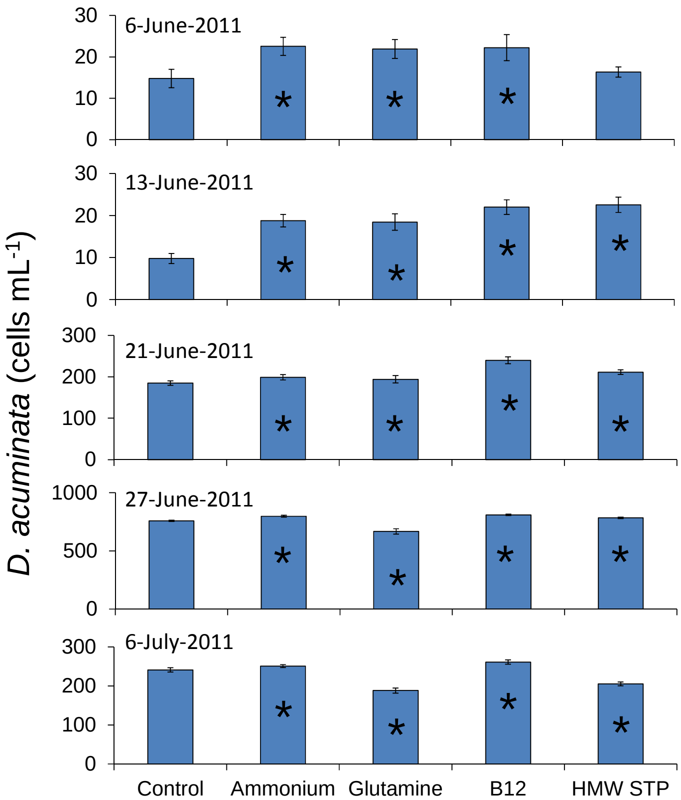


Figure 17. *Dinophysis acuminata* densities (cells mL^{-1}) following nutrient amendment experiments conducted with Northport Bay water in the spring of 2011. Bars are means while error bars represent SD of triplicate measurements. Asterisks denote treatments significantly different from the control.

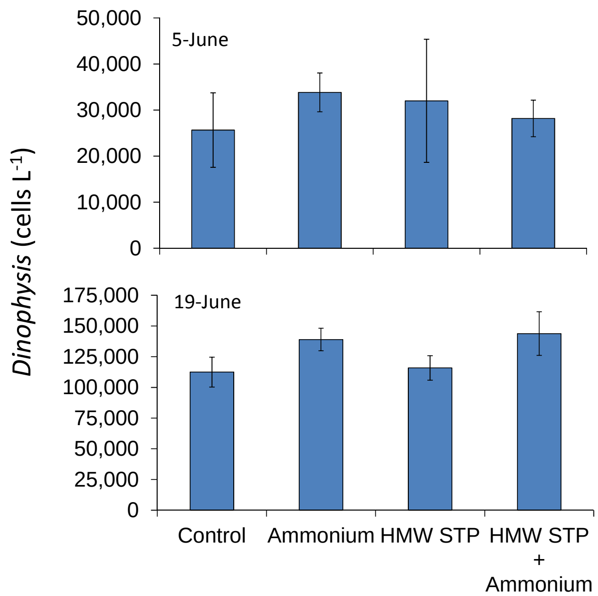


Figure 18. *Dinophysis* densities (cells L^{-1}) following nutrient amendment experiments conducted with Northport Bay water in the spring of 2012. Bars are means while error bars represent SD of triplicate measurements.

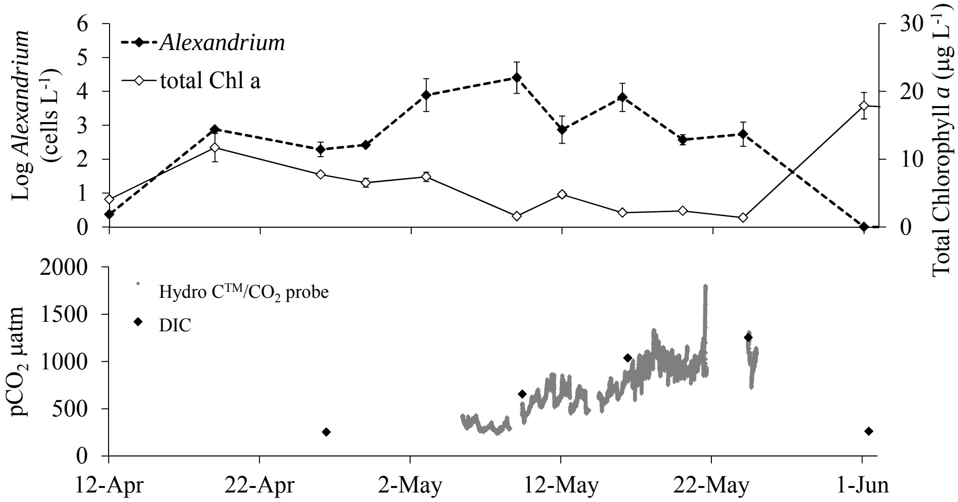


Figure 19. Top panel: Log *Alexandrium* densities (cells L^{-1}) and total chlorophyll *a* (µg L^{-1}). Bottom panel: pCO_2 (µatm) as measured by a HydroC™/CO_2 (Contros) probe that was deployed in Northport Harbor, NY, USA during 2011 and discrete dissolved inorganic carbon (DIC) samples used to ground truth the probe.

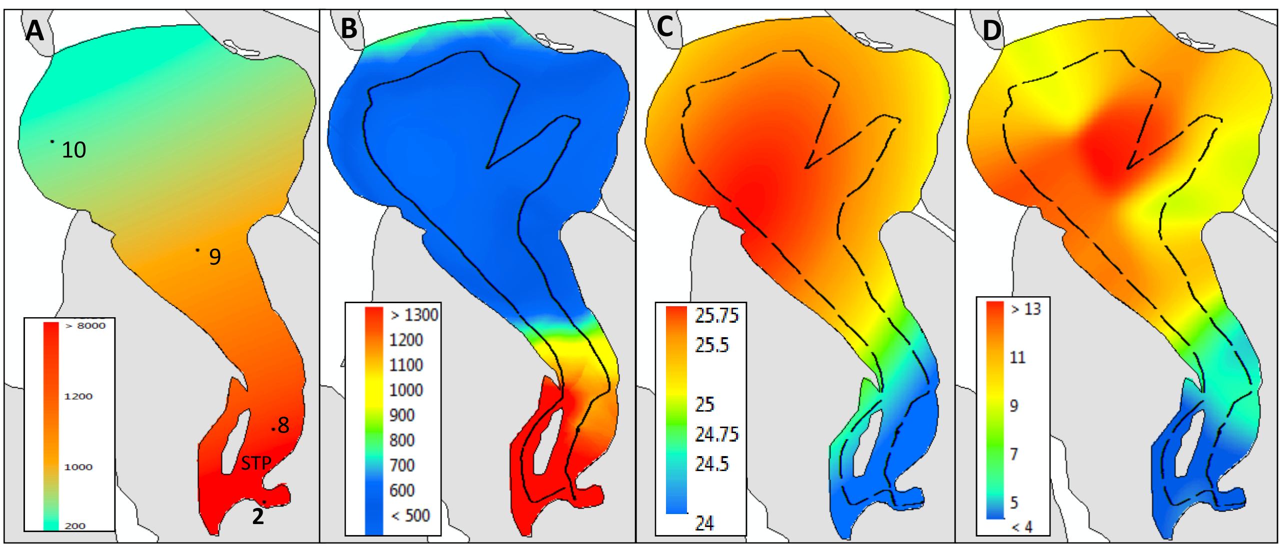


Figure 20. Heat maps of A) *Alexandrium* densities (cells L^{-1}), B) pCO_2 (µatm) as measured by a HydroC™/CO_2 (Contros) probe, and C) salinity (psu) and D) chlorophyll *a* (µg L^{-1}) as measured by a YSI 6920v2 probe, from a horizontal transect conducted in Northport Bay in May of 2012. Maps were created using geostatistical analyst in ARC GIS 10. Points in (A) represent sampling sites where lines in (B-D) represent multiple data points taken in close proximity via probes. STP indicates the location of the Scudder Beach Sewage treatment plant outflow.

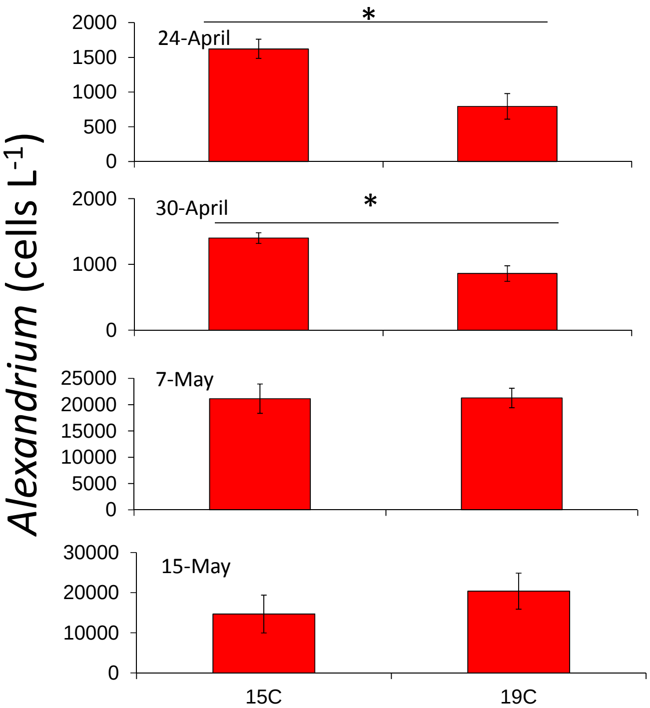


Figure 21. *Alexandrium* densities (cells L^{-1}) following experiments assessing the effects of different temperatures on the growth of *Alexandrium*. Northport Bay water was incubated in chambers with temperatures of 15ºC and 19ºC.

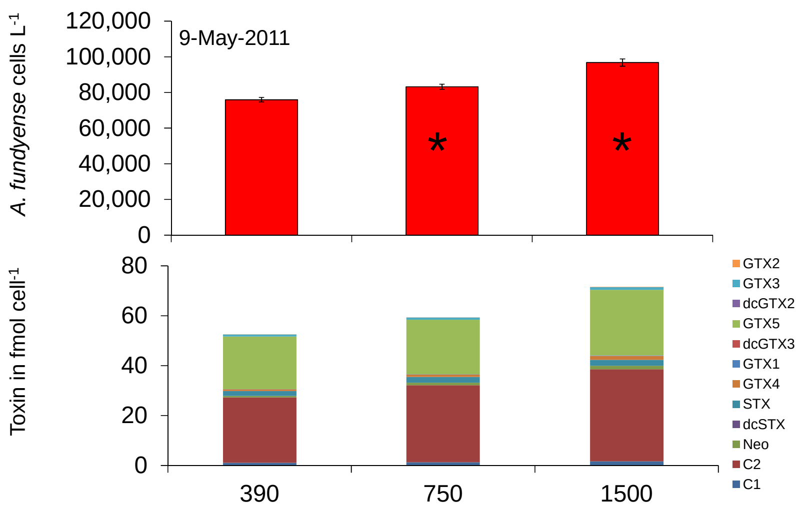


Figure 22. Effects of varying levels of CO_2 on *Alexandrium* densities and toxicity during short-term field experiments using water from Northport Bay. Bars are means while error bars represent the SD of triplicate measurements.

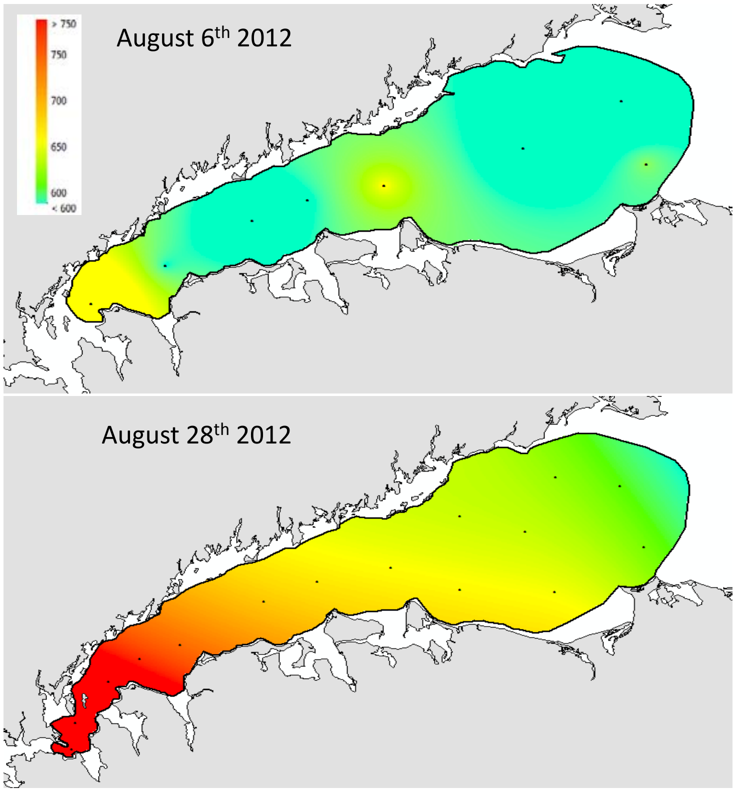


Figure 23. Heat maps of cruises conducted during August 2012 to assess the spatial distribution of pCO_2 across the eutrophic gradient of Long Island Sound. Black circles indicate stations where vertical profiles of pCO_2 were measured using the Hydro C^{TM}/CO_2 probe (Contros). Data in heat maps represents pCO_2 µatm at 2m.

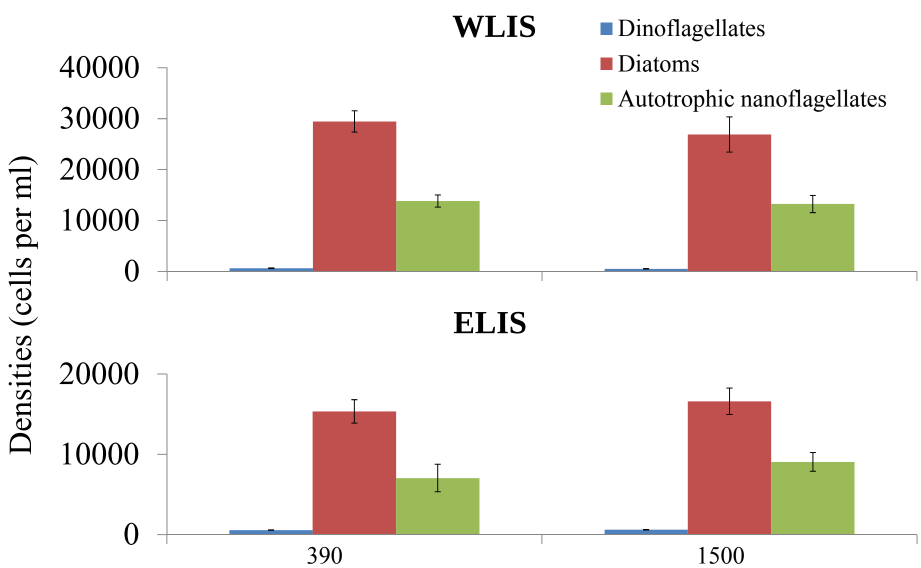


Figure 24. Effects of varying levels of CO_2 on phytoplankton communities from eastern and western Long Island Sound during short-term field experiments. Bars are means while error bars represent the SD of triplicate measurements.

TABLES:

Table 1. The highest observed *Alexandrium* cell densities (cells L^{-1}) found at each sampling location from 2007-2012. The number of samples collected at each location = the number of times each location was sampled.

Region	Location	date of highest *Alexandrium* densities	*Alexandrium* (cells L^{-1})	# of samples collected at location	# of positive samples	% of positive samples
Connecticut	Holly Pond	20-May-09	4	5	1	20
Connecticut	Norwalk Harbor	4-Jun-09	11	6	1	17
Connecticut	Sherwood Millpond	n/a	0	5	0	0
Connecticut	Black Rock	n/a	0	6	0	0
Connecticut	Branford Harbor	4-Jun-09	6	6	1	17
Connecticut	North Cove	n/a	0	6	0	0
Connecticut	Palmers Cove	25-Jun-09	4	5	2	40
Connecticut	Mumford Cove	6-May-10	8	7	2	29
Connecticut	Mystic Harbor	18-Jun-09	32	10	4	40
New York	Purchase	25-May-10	18	6	1	17
North shore Long Island	Little Neck Bay	19-May-10	2	5	1	20
North shore Long Island	Manhasset Bay	25-May-09	12	6	1	17
North shore Long Island	Hempstead Harbor	18-Apr-12	5	11	3	27
North shore Long Island	Oyster Bay Harbor	20-May-11	76	14	4	29
North shore Long Island	Cold Spring Harbor	25-May-09	44	11	3	27
North shore Long Island	Northport Harbor - Northport-Huntington Bay system	16-May-08	1,199,567	146	108	74
North shore Long Island	Centerport Harbor- Northport-Huntington Bay system	23-May-08	7,166	26	10	38
North shore Long Island	Northport Bay- Northport-Huntington Bay system	26-May-08	31,675	25	19	76
North shore Long Island	Huntington Bay- Northport-Huntington Bay system	26-May-08	28,178	24	19	79
North shore Long Island	Huntington Harbor- Northport Bay system	23-May-08	24,850	34	25	74
North shore Long Island	Long Island Sound Station 7 (outside Northport-Huntington Bay system)	26-May-08	8,244	8	7	88
North shore Long Island	Nissequogue River	n/a	0	5	0	0
North shore Long Island	Stony Brook Harbor	n/a	0	10	0	0
North shore Long Island	Port Jefferson	16-May-08	201	37	10	27
North shore Long Island	Mount Sinai Harbor	31-May-12	3	10	1	10
North shore Long Island	Mattituck creek system	2-Jul-09	84,700	62	34	55
North shore Long Island	Long Island Sound Station 14 (Orient Point)	4-Jun-09	21	1	1	100
North shore Long Island	Long Island Sound Station 15 (Gardiners Bay)	4-Jun-09	113	1	1	100
New York Peconics	Meetinghouse Creek	23-Apr-09	19,868	43	27	63
New York Peconics	Reeves Bay	26-Apr-12	2,942	10	7	70
New York Peconics	Peconic River	9-May-08	615	4	4	100
South Shore Long Island	Old Fort Pond	29-Apr-08	414	12	7	58
South Shore Long Island	Weesuck Creek	27-Apr-11	49,042	19	17	89
South Shore Long Island	Quantuck	22-Apr-08	1,902	18	14	78
South Shore Long Island	Beaverdam Creek	15-Apr-08	228	6	4	67
South Shore Long Island	Seatuck	28-May-08	15	12	3	25
South Shore Long Island	Harts Cove	30-Apr-08	9	6	2	33
South Shore Long Island	Forge River	30-Apr-08	11,023	11	8	73
South Shore Long Island	Patchogue	25-Apr-12	14	8	4	50
South Shore Long Island	Belport	10-May-12	78	8	4	50
South Shore Long Island	Bayshore	14-Apr-10	32	8	6	75
South Shore Long Island	Jamaica Bay	19-May-10	102	2	2	100
South Shore Long Island	East Bay	10-May-10	35	3	2	67
South Shore Long Island	Middle Bay	10-May-10	138	2	2	100
South Shore Long Island	Bay Park (Hewlett Bay)	10-May-10	788	2	2	100
South Shore Long Island	South Oyster Bay	12-Mar-12	8	6	2	33
East End Long Island	Orient Harbor	17-May-12	8	3	1	33
East End Long Island	Greenpoint Harbor	n/a	0	5	0	0
East End Long Island	Haywater Cove	2-May-12	1,736	5	3	60
East End Long Island	Lake Montauk	3-May-12	2	5	1	20
East End Long Island	Three Mile Harbor	n/a	0	5	0	0
East End Long Island	Sag Harbor Cove	17-Apr-12	3,495	5	2	40
East End Long Island	West Neck Bay	3-May-12	9	5	1	20
East End Long Island	North Sea Harbor	27-Apr-12	4	5	1	20
East End Long Island	Cold Spring Pond	8-May-12	2	5	1	20

Table 2. The highest observed *Dinophysis* cell densities (cells L^{-1}) found at each sampling location from 2008-2012. The number of samples collected at each location = the number of times each location was sampled.

Region	Location	date of highest *Dinophysis* densities	Dinophysis (cells L^{-1})	# of samples collected at location	# of positive samples	% of positive samples
North shore Long Island	Hempstead Harbor	31-May-12	6,944	8	7	88
North shore Long Island	Oyster Bay Harbor	14-Aug-12	490	9	5	56
North shore Long Island	Cold Spring Harbor	15-Jun-12	22,274	9	8	89
North shore Long Island	Northport Harbor - Northport-Huntington Bay system	27-Jun-11	1,266,000	136	95	70
North shore Long Island	Northport Bay- Northport-Huntington Bay system	27-Jun-11	168	4	4	100
North shore Long Island	Huntington Bay- Northport-Huntington Bay system	27-Jun-11	252	5	5	100
North shore Long Island	Huntington Harbor- Northport Bay system	19-Jun-12	3,934	15	10	67
North shore Long Island	LIS- outside of Northport Bay system	16-Jun-11	252	3	3	100
North shore Long Island	Stony Brook Harbor	13-Jun-12	84	9	5	56
North shore Long Island	Port Jefferson	3-June-2012, 2-July-201	56	9	4	44
North shore Long Island	Mount Sinai Harbor	18-May-12	98	9	7	78
North shore Long Island	Mattituck creek system	2-May-12	8,344	18	14	78
New York Peconics	Meetinghouse Creek	2-May-12	2,123,000	28	22	79
New York Peconics	Reeves Bay	31-May-12	63,378	15	14	93
South Shore Long Island	Old Fort Pond	10-Jul-12	1,456	10	8	80
South Shore Long Island	Weesuck Creek	27-Apr-12	1,274	19	15	79
South Shore Long Island	Quantuck	11-May-12	1,554	14	10	71
South Shore Long Island	Penniman Creek	5-June-12, 6-July-12	42	7	4	57
South Shore Long Island	Seatuck	6-Jun-12	13,944	10	8	80
South Shore Long Island	Forge River	25-Apr-12	24,080	10	6	60
South Shore Long Island	Patchogue	24-May-12	196	10	6	60
South Shore Long Island	Belport	25-Apr-12	1,134	10	8	80
South Shore Long Island	Bayshore	11-May-12	6,006	10	8	80
South Shore Long Island	South Oyster Bay	12-Apr-12	504	10	6	60
South Shore Long Island	Bay Park	24-May-10	6,000	23	5	22
South Shore Long Island	East Bay	9-Jun-10	4,000	8	4	50
South Shore Long Island	Middle Bay	9-Jun-10	4,000	8	3	38
South Shore Long Island	Jones Beach Inlet	9-Jun-10	24,000	8	4	50
East End Long Island	Orient Harbor	10-Jul-12	154	7	7	100
East End Long Island	Greenpoint Harbor	6-Apr-12	224	9	7	78
East End Long Island	Haywater Cove	2-May-12	966	9	7	78
East End Long Island	Lake Montauk	27-Jun-12	2,758	9	6	67
East End Long Island	Three Mile Harbor	27-Jun-12	980	9	5	56
East End Long Island	Sag Harbor Cove	17-May-12	11,060	9	7	78
East End Long Island	West Neck Bay	17-May-12	308	9	3	33
East End Long Island	North Sea Harbor	16-May-12	1,064	8	6	75
East End Long Island	Cold Spring Pond	29-May-12	196	10	8	80

Table 3. Okadaic acid congener and pectenotoxin concentrations (ng g^{-1}) measured in shellfish collected from the Northport-Huntington Bay complex located in NY, USA. *Mytilus edulis* were hung in bags for monitoring purposes, whereas *Mya arenaria* and *Geukensia demissa* were collected. Samples were hydrolyzed therefore OA and DTX1 represent both free acids and esters. <dl indicates samples were below detection limit. Numbers in bold indicate samples above the FDA action level. OA=okadaic acid, DTX= dinophysistoxins, PTX= pectenotoxins.

Date	Shellfish Collection site	Location name	Longitude	Latitude	Shellfish species	OA	DTX1	DTX2	total OA congeners	PTX
28-Jun-2010	S3	Woodbine Marina	-73.35360	40.89880	*Mytilus edulis*	39	13	<dl	52	0.4
28-Jun-2010	S4	Northport Bay	-73.37560	40.91640	*Mytilus edulis*	74	41	<dl	115	7
20-Jun-2011	S7	Huntington Harbor	-73.41690	40.88840	*Mytilus edulis*	93	50	<dl	143	9
28-Jun-2011	S3	Woodbine Marina	-73.35360	40.89880	*Mytilus edulis*	790	455	<dl	**1245**	115
6-Jul-2011	S5	Asharoken	-73.35440	40.92150	*Mytilus edulis*	107	58	<dl	**165**	5
6-Jul-2011	S6	Huntington Bay	-73.43030	40.91650	*Mytilus edulis*	24	13	<dl	37	3
7-Jul-2011	S1	South Scudder Beach	-73.35717	40.89211	*Mya arenaria*	660	297	<dl	**957**	66
7-Jul-2011	S2	North Scudder Beach	-73.35739	40.89311	*Mya arenaria*	758	331	<dl	**1089**	42
7-Jul-2011	S2	North Scudder Beach	-73.35739	40.89311	*Geukensia demissa*	818	319	<dl	**1137**	71

NYSG Completion Report Instructions & Required Format

Please include the following information for your project. The text of this report should be at least 5-8 pages and be composed for an audience of your peers. Other formats, or reports with incomplete sections, will not be accepted. The expectation is that information or material will be provided under each section.

Report Written By: _Caitlin Young________ Date: ___2-10-14_________

A. Project Number and Title: R/CTP-44-NYCT '*Sources and Fate of Nitrogen in North Shore Embayment's*

Project Personnel:
Dr. Gilbert Hanson, Principal Investigator
Dr. Teng-Fong Wong, Co- Principal Investigator
Caitlin Young, NYSG Scholar
Neal Stark, Senior Research Support Specialist
Josephine Durand, Graduate Research Assistant
Ron Paulsen, Suffolk County Department of Health Services Personnel
Jonathan Wanlass, Suffolk County Department of Health Services Personnel

B. Project Results::

C1. Meeting the Objectives:

Objective 1. Following a grid of transects, as shown in figure 1, use a combination of temperature and resistivity measurements to estimate the amount of groundwater entering Port Jefferson (PJH) and Stony Brook Harbors (SBH) using the Trident probe and SuperSting resistivity.

1. Results: Resistivity and Trident transects were performed in SBH (April 2011, August 2011) and in PJH (March-April 2012). Overall Trident surveys indicated a pattern spatially heterogeneous diffuse freshwater discharge along shoreline. Offshore locations did not exhibit freshening at the sampling depth of 60cm beneath the sediment water interface (see attached report, chapters 3 and 5).
   Resistivity transects in SBH and PJH revealed previously unknown freshwater plumes extending beneath the harbors for a distance of tens of meters offshore (see attached manuscript draft from Durand et al). These plumes are capped by a layer of marine mud which acts as an aquitard to offshore SGD. Inland hydraulic gradient due to steep topography of the embayment topography drives freshwater offshore.

Objective 2. Identify major plumes of groundwater entering Port Jefferson Harbor and Stony Brook Harbor. Sample within those plumes using a Trident probe. Use barge mounted Geoprobe to obtain sediment cores

2. Results: Using data from results of Objective 1, major groundwater plumes were identified in SBH and PJH. These plumes were the sites of further investigation, including porewater sampling. Due to sediment compaction, we were unable to obtain sediment cores offshore. Instead, cores were obtained in the intertidal and subtidal zones at SBH and analyzed for sediment composition (see chapter 3 in attached dissertation for full results). In PJH, a barge mounted Geoprobe was used to install permanent multi-level sampling wells that were used to take samples along two shore perpendicular transects; one located at Centennial Beach and one located in Belle Terre.

Objective 3. Identify any variations in SGD in the spring and fall periods following periods of highest and lowest recharge to the groundwater and how these impact overall nitrogen loading to the harbors.

3. Results: Variations in spring and fall periods were examined in SBH during 2011 and in PJH during 2012. Nitrate concentration data sets from May and October were analyzed by t-test to determine if a significant seasonal difference exists. October concentrations were found to be significantly higher ($p=0.007$), with an average concentration of 310µmol L^{-1}, as compared to 200µmol L^{-1} during May. During May maximum nitrate values are observed in at the high tide point but in October maximum nitrate values are observed in the fresh water zone at the base of the intertidal marsh, just prior to discharge. See attached dissertation chapter 3 'DENITRIFICATION AND NITRATE BIOGEOCHEMISTRY IN A SUBTERRANEAN ESTUARY OF STONY BROOK HARBOR' (figures 16d and 18d for an extended discussion of seasonal trend results)

    An additional study was performed to investigate nutrient dynamics in the subterranean estuary over two spring neap tidal cycles. This study found salinity and oxygen was consistently stratified over the spring-neap period, but nitrate and phosphate concentrations varied between the two periods. A large terrestrial hydraulic gradient resulted in a stable salinity depth profile despite daily two meter tidal oscillations. Fresh groundwater contained high concentrations of nitrate, averaging 6.3±2.7mgL-1NO3--N (450±193µmol L-1) at a depth of 9.1m. Maximum inorganic phosphate concentrations, averaged 0.13mgL-1 PO4-3-P (4.2µmol L-1), at sampling depth 1.8m. Mass balance models were used to estimate fresh and saline fractions of discharge during spring and neap tide periods. Spring tide discharge was estimated at 1.3 L min-1m-1 and 48.0 L min-1m-1 for freshwater and saltwater respectively. Neap tide discharge was estimated at 6.5 L min-1m-1 and 28.0 L min-1m-1 for fresh and saltwater respectively. These differences in salt vs fresh water factions of SGD resulted in water table over height during spring tide. Consequently, water-table over height causes migration of the freshwater discharge point along the beach face resulting in variation of nutrient concentrations. See attached dissertation chapter 2 'NUTRIENT DYNAMICS IN A SUBTERRANEAN ESTUARY OVER TWO SPRING-NEAP TIDAL CYCLES' for full report on this study.

Objective 4. Use piezometers to collect samples in the subterranean estuary. Transects will be 8-10 meters long, consisting of 10-15 sampling points with depth into the sediment ranging from 0-2m. Use these samples to define zones of salinity. Collect sub-bottom pore water samples to characterize the *in situ* nitrogen concentrations from the seawater sediment interface to a maximum depth of 60cm.

4. A total of 7 piezometer transects were completed during this study; 3 in SBH, 1 in Setauket Harbor and 3 in PJH. These samples were used to examine spatial and seasonal trends in nitrate discharge into three Long Island Sound Embayments. In SBH the coastal aquifer consisted of an upper saline plume, underlying freshwater zone and deep saline zone at the two piezometer transect locations. Denitrification rates were found to be spatially heterogeneous, with the percent of nitrate denitrified ranging 0 to 35% at low tide discharge points and up to 47% during transport to the embayment floor in offshore discharge zones. See attached dissertation chapter 3 'DENITRIFICATION AND NITRATE BIOGEOCHEMISTRY IN A SUBTERRANEAN ESTUARY OF STONY BROOK HARBOR' for a full discussion of these results. In PJH, piezometer profiles at Centennial beach revealed a freshwater discharge zone that outcrops at the beach surface at low tide. The lack of upper saline plume prevents incorporation of dissolved organic carbon and therefore reduces denitrification potential in this portion of the coastal aquifer. A second site in PJH was located in Belle Terre.

At this site the existence of an upper saline plume produced maximum denitrification rates in shallow sediments of the low tide zone.

Sub bottom porewater samples were collected in PJH and used in conjunction with a ^{222}Rn survey to estimate nutrient flux to the harbor during spring discharge time period. This study revealed three nitrogen input mechanisms to PJH via SGD; in the southern end of the harbor high porewater nitrate concentrations coincide with high ^{222}Rn concentrations, linking SGD with nitrate inputs that originate upgradient in the shallow aquifer. Along the east and north east harbor shoreline, low nitrate and high ^{222}Rn values were the result of harbor water circulation through sediments in the intertidal zone in a low density housing area, which limited the amount of nitrate entering the harbor from this portion of the coastal aquifer. Finally, the western shore of the harbor exhibited trends of low ^{222}Rn and low nitrate concentrations, indicating SGD was not a significant process along the western shore of PJH. In conclusion, nitrate inputs from SGD_{total} were estimated to be 800mol d^{-1} (11kg d^{-1}). Although this estimate is restricted to shoreline discharges of nitrate and does not account for input entering the harbor through offshore mud sediments, it is similar to the nitrogen input to the harbor from the Port Jefferson Sewage Treatment Plant (STP), which currently averages 12.2 kg N d^{-1}. Estimates of nitrate input to Port Jefferson Harbor (this study) are ~1.2% of total SGD nitrogen inputs to Long Island Sound from all of Suffolk County (Scorca and Monti, 2001). For a full description of this study, including tables and figures, please see chapter 5 in the attached dissertation ‘EMBAYMENT SCALE ASSESSMENT OF SUBMARINE GROUNDWATER DISCHARGE NUTRIENT LOADING TO PORT JEFFERSON HARBOR, LONG ISLAND NY’.

Objective 5. Analyze all samples for nitrogen species, phosphate and major electron donors. Use this information to determine the major nitrate attenuation/generation processes.

5. Results: This process was performed throughout the study. See attached dissertation chapters 2-5 for a detailed report of nitrogen attenuation rates and mechanisms for SBH, PJH and Setauket Harbor

Objective 6. Integrate SGD survey data and geochemical data using GIS to generate maps for use by relevant authorities to create effective watershed management policies.

6. Results: SGD survey and geochemical data was made into maps for PJH as described above in dissertation chapter 5, ‘EMBAYMENT SCALE ASSESSMENT OF SUBMARINE GROUNDWATER DISCHARGE NUTRIENT LOADING TO PORT JEFFERSON HARBOR, LONG ISLAND NY’. Given the restricted nature of piezometer transects, it was determined that data from SBH sampling was not applicable to harborwide mapping of SGD.

Objective7. Apply study results to existing nitrogen loading models and distribute study results via publication and at local and national conferences.

7. Results: Study results have been presented at numerous national and local conferences;

   2014: ‘N_2O Formation Mechanisms in Sandy Unconfined Coastal Aquifers’ Goldschmidt Geochemistry Conference June 8-13

   2013: ‘Distribution of submarine groundwater discharge into Port Jefferson Harbor, Long Island Sound, NY’ American Society of Limnology and Oceanography, February 18-22

2012: 'Nitrogen transformations during oxic SGD in Stony Brook Harbor, NY American Geophysical Union, December 3-7

2012: 'Fate of nitrogen during oxic submarine groundwater discharge into Stony Brook Harbor, New York' Goldschmidt Geochemistry Conference, June 24-29

2011: 'Groundwater nitrate attenuation during transport through a subterranean estuary in a Long Island Sound embayment' American Geophysical Union, Dec 5-9

C2. Scientific Abstract: Long Island Sound experiences periods of hypoxia attributed to eutrophication, but the magnitude of nitrogen contributed to surface water via submarine groundwater discharge (SGD) entering Long Island's north shore embayments is not well characterized. The coastal aquifer, where fresh groundwater mixes with saline coastal water is termed the subterranean estuary (STE). Advective flow combined with sharp salinity and dissolved oxygen gradients make the STE a zone of intense geochemical cycling. However, the fate of nitrogen during transit through Long island embayment STEs is not well understood, particularly how sediment heterogeneity influences nitrogen attenuation in discharge zones.

Nitrate attenuation mechanisms, principally denitrification, were investigated in three Long Island north shore embayments; Stony Brook Harbor, Setauket Harbor and Port Jefferson Harbor. In Stony Brook Harbor an investigation of freshwater nitrate dynamics over two spring-neap tidal cycles found oscillations in depth stratified nitrate concentrations. Calculation of fresh fraction discharge revealed that water table over-height is responsible for these oscillations, which result from shore perpendicular movement of the coarse sediment freshwater discharge point.

High resolution sampling of STE porewater from Stony Brook Harbor and Setauket Harbor revealed discharge of freshwater continues for tens of meters offshore, which results in two zones of nitrogen removal. When SGD discharges into surface water near low tide through coarse-grain sand or marsh sediments, denitrification rates are 15 - 50% lower than when SGD passes through into a fine grain sediment layer offshore.

In Port Jefferson Harbor, results from a combined shallow porewater nitrate concentration and geochemical tracer (^{222}Rn) study indicate SGD accounts for similar nitrogen flux to surface water as direct inputs from a local sewage treatment plant. Overall, embayment scale sediment heterogeneity is positively correlated with availability of dissolved organic carbon, which in turn controls the extent of microbially mediated denitrification found in each of the studied embayments.

C3. Problems Encountered:

Major difficulties were as follows

a) Data Collection: Due to full tidal flushing, collection of SGD rate data in Setauket harbor was not possible. A number of methods were tried, including manual seepage meters, automatic seepage meters and ^{222}Rn measurements. The conclusion was that these methods did not provide reliable estimates of SGD for this harbor. Therefore, we used porewater Cl^- profiles in an advective-diffusion model to estimate SGD rates for three different zones of the harbor. See attached dissertation chapter 4 'NUTRIENT RELEASE FROM A GROUNDWATER FED TIDAL FLAT IN SETAUKET HARBOR, LONG ISLAND NY' for a full description of porewater modeling results.
b) Sample analysis: Results from PJH were delayed by approximately 1 year due to slow sample analysis. Samples for dissolved N_2/Ar measurements were sent to UC Davis Stable Isotope Laboratory for analysis, with an initial turn around estimate of 6 weeks. Unfortunately

results were not received for ~1year. Data was received at the end of December, 2013. Consequently, this final report does not include all data analysis, as denitrification estimates were not possible without N_2/Ar data. In lieu of this data, we instead used a coupled ^{222}Rn and porewater nitrogen concentration survey to examine how SGD transports nitrate to surface water in PJH. Results from this work are detailed in chapter 5 of the attached dissertation 'EMBAYMENT SCALE ASSESSMENT OF SUBMARINE GROUNDWATER DISCHARGE NUTRIENT LOADING TO PORT JEFFERSON HARBOR, LONG ISLAND NY'

C4. New Research Directions:

Two additional research directions were pursued during this project.

1. Radon concentration mapping of surface water in conjunction with shallow porewater sampling: We wished to directly address the proposed installation of seepage pits to redirect sewage away from the Sewage Treatment Plant on Stony Brook University campus (SCSD no.21), which was proposed by Suffolk County legislature to lower the total maximum daily limits (TMDLs) of nitrogen entering surface body waters. It was proposed that the seepage pits would alleviate nitrogen loading to surface waters. We wished to test this hypothesis by examining spatially distributed nitrogen inputs to PJH.

   Results from this new research direction are detailed in 'EMBAYMENT SCALE ASSESSMENT OF SUBMARINE GROUNDWATER DISCHARGE NUTRIENT LOADING TO PORT JEFFERSON HARBOR, LONG ISLAND NY'. The primary conclusion is that SGD carries ~11kg NO_3^-/day to PJH during shoreline discharge, which is similar to loading from the existing sewage treatment plant outfall (~12.2kg-N/d). Therefore, the proposed seepage pits are unlikely to reduce the amount of nitrogen entering the harbor, but will instead delay nitrogen loading in accordance with groundwater travel time.

2. Thermal infrared mapping of surface waters. During this project, we investigated the feasibility of thermal infrared mapping to distinguish areas of diffuse SGD. The data was used in NASA and SeaGrant proposal calls. The result was funding for NYSG Proposal SG14014 '"*The role of submarine groundwater discharge (SGD) in promoting hypoxia in Smithtown Bay*" and for NASA graduate student scholarship (J.Tamborski)

C5. Interactions:

C6. Presentations and Publications:

Presentations:

Young, C.R, G.N Hanson. 2014 'N_2O Formation Mechanisms in Sandy Unconfined Coastal Aquifers' Goldschmidt Geochemistry Conference, Sacramento CA, 8-13 June

Young, C.R, J. Tamborski, A.D Rogers, G.N Hanson 2013: 'Distribution of submarine groundwater discharge into Port Jefferson Harbor, Long Island Sound, NY' American Society of Limnology and Oceanography, New Orleans, LA 18-22 February. Presentation

Young, C.R, J. Durand, G.N Hanson, 2012: 'Nitrogen transformations during oxic SGD in Stony Brook Harbor, NY American Geophysical Union, San Francisco, CA 3-7 December. Poster

Young C.R and G.N Hanson 2012: 'Fate of nitrogen during oxic submarine groundwater discharge into Stony Brook Harbor, New York' Goldschmidt Geochemistry Conference, Montreal, Canada 24-29 June. Poster.

Young, C.R. and G.N. Hanson 2011: 'Groundwater nitrate attenuation during transport through a subterranean estuary in a Long Island Sound embayment' American Geophysical Union, San Francisco, CA 5-9 December. Poster.

NYSG Completion Report Instructions & Required Format

D. Accomplishments: Complete the following sub-sections:

D1. Impacts & Effects:

This project is expected to have significant impact on decision making for regional land management organizations and government bodies. Overall, results from this study show that Long Island groundwater, which is known to contain high concentrations of nitrate, does not undergo more than 35% denitrification during submarine groundwater discharge in areas where discharge rates are in excess of 25cm/d. Seasonal trends show that denitrification rates are significantly lower in spring than in fall, which may affect coastal eutrophication as nutrients are added during the bloom phase of surface water phytoplankton life cycle.

Findings from this project indicate that it the installation of sewage seepage pits to offset surface water discharges from a sewage treatment plant does not provide a positive environmental impact. Instead, nitrogen added to the surficial aquifer will ultimately impact Long Island Sound harbors via SGD. Results from this study should be disseminated to state and local environmental regulation offices in order to provide data for sewage treatment plant decision making.

D2. Scholar(s) & Student(s) Status:

NYSG Scholar Caitlin Young- Scholar was awarded a Thesis Completion Award (TCA) by SeaGrant for the summer of 2013. Thesis defense was completed in November of 2013, and graduation awarded in December of 2013. Post-graduation, the NYSG scholar is employed as a Postdoctoral Associate in the Geological Sciences department at University of Florida, in the research group of Dr. Jonathan Martin.

Non-financially supported students

1. Josephine Durand: Contributed to collection and processing of geophysical data. She has an anticipated graduation date of August 2014. J.Durand will be publishing at least one paper detailing the geophysical results on the STE investigation at SBH (see attached). She will additionally be co-authoring the 2 of the remaining papers that result from R-CTP-44.
2. Joseph Tamborski: Contributed to collection of porewater data in PJH, thermal infrared data in PJH and ^{222}Rn surveys. J.Tamborski will be co-authoring 1 of the papers that result from R-CTP-44. He is currently supported by SG14014.

D3. Volunteers: Provide information about any volunteers (citizens or students) who worked on the project. Indicate their activity and amount of time (hours) they participated.

Michael Thorpe- Graduate student in Geosciences. Assisted with collection of porewater samples for trace metal analysis in PJH. Approximate amount of time spent on project was 6 months.

Tsou ShuHsing- Undergraduate student in Marine sceinces assisted in collection of seepage meter data in PJH in May and July of 2012.

Adrien Pernet- Undergraduate exchange student from France. Assisted with sediment core sampling and processing of sediment potential denitrification rates in SBH. Amount of time spent on project was 6 weeks in July-August of 2011.

D4. Patents: N/A

E. Stakeholder Summary:

'Sources and Fate of Nitrogen in North Shore Embayment's ' was designed to gain a better understanding of how nitrogen, a nutrient that can cause coastal eutrophication, is transported through coastal aquifers during sumarine groundwater discharge (SGD) to Long Island's north shore embayments. The project investigated three major harbors of Long Island Sound; Stony Brook Harbor (SBH), Setauket Harbor and Port Jefferson Harbor (PJH). In each harbor a spatial survey was conducted to determine areas of SGD. Results from these spatial surveys indicated that highest rates of SGD are found at or near the low tide zone, with rates ranging 25 cm/d to 102cm/d, and areas with the greatest SGD is found on the western shore of SBH and the eastern shore of PJH. This is likely due to large topographic inland relief of these two shorelines, which generates higher hydraulic head in the surficial aquifer, driving groundwater down gradient towards the coast. In all three harbors SGD was also observed offshore, through marine mud cap that covers the harbor bottoms at a maximum distance of 60m from mean low tide. This is the first investigation to observe this type of SGD in Long Island Sound embayments, where rates through the mud cap ranged 0 to 2cm/d, significantly lower than rates measured in permeable sediments at low tide.

Nitrogen transformations during SGD were examined in all three harbors. Major findings for Stony Brook Harbor indicate that denitrification attenuates less than 35% of the nitrate discharging in the low tide zone, but nitrate undergoes ~47% denitrification during transport to the base of the mud cap in the offshore zone. Nitrate discharges were higher during spring than fall, which may provide an excess of nutrients to surface waters during the time of algal spring bloom. In Setauket Harbor, modeled nutrient consumption and production indicated the harbor was a net sink for freshwater sourced nitrate, but was a net source of dissolved organic carbon. This was due to reducing conditions in the central portion of the harbor, which promoted denitrification during freshwater transport to the base of the mud layer. Finally, in Port Jefferson Harbor nutrient measurements were coupled with dissolved ^{222}Rn concentrations (a SGD geochemical tracer) which provided insight into nitrogen loading from the entire coastline. This data was compared to direct nitrogen inputs from a local sewage treatment plant that discharges to Port Jefferson Harbor. It was determined that nitrogen inputs from shoreline SGD were approximately equivalent (11kg NO_3^--N /d) to average reported inputs from the sewage treatment plant (12.2kg N/d). These results have direct implcations for land management planning, particularly proposals to install inland sewage seepage pits as an alternative to sewage treatment plant direct discharge. Given that shoreline SGD currently provides an equivalent amount of nitrogen as direct inputs, the addtion of nitrogen to the shallow aquifer is unlikely to lower the total amount of nitrogen input to Port Jefferson Harbor. Instead, additions of nitrogen to groundwater will most likely increase the SGD nitrogen loading to Port Jefferson Harbor.

In conclusion, this study has demonstrated the importance of submarine groundwater discharge to surface water nitrogen loading in Long Island Sound embayments. We found limited denitrification along shorelines with high discharge rates, indicating the coastal aquifer does not fully attenuate groundwater derived nitrogen. Results from this study highlight the importance of land based nitrogen loading mitigation, as in most cases the coastal aquifer does not act as a zone of denitrification.

F. **Pictorial:** Please see attached the completed dissertation of SeaGrant scholar Caitlin Young. See also attached manuscript draft by Durand, Young, Hanson and Wong.

CONNECTICUT SEA GRANT PROJECT REPORT

Please complete this progress or final report form and return by the date indicated in the emailed progress report request from the Connecticut Sea Grant College Program. Fill in the requested information using your word processor (i.e., Microsoft Word), and e-mail the completed form to Dr. Syma Ebbin syma.ebbin@uconn.edu, Research Coordinator, Connecticut Sea Grant College Program. Do NOT mail or fax hard copies. Please try to be address the specific sections below. If applicable, you can attach files of electronic publications when you return the form. If you have questions, please call Syma Ebbin at (860) 405-9278.

Please fill out all of the following that apply to your specific research or development project. Pay particular attention to goals, accomplishments, benefits, impacts and publications, where applicable.

Project #: **R/CTP-45-CTNY** Check one: [✓] Progress Report [ ] Final report

Duration (dates) of entire project, including extensions: From [4/1/11] to [3/31/14].

Project Title or Topic: **Systematic Evaluation of Nitrogen Removal by BMPs in the Long Island Sound Watershed**

Principal Investigator(s) and Affiliation(s):
1. Shimon Anisfeld / School of Forestry & Environmental Studies, Yale University
2. Gaboury Benoit / School of Forestry & Environmental Studies, Yale University

A. COLLABORATORS AND PARTNERS: *(List any additional organizations or partners involved in the project.)*

The South Central Connecticut Regional Water Authority and the Town of Woodbridge have granted us access to their sites.

B. PROJECT GOALS AND OBJECTIVES:

1. Evaluate the effectiveness of constructed wetlands and wet ponds in removing nitrogen (N) from stormwater in the LIS watershed.
2. Evaluate how N removal is affected by each of these factors: influent N concentration; season; water residence time; water infiltration; soil/substrate characteristics (texture, organic matter content); vegetation cover
3. Provide recommendations on design of stormwater ponds/wetlands for optimal N removal in the LIS watershed.

C. PROGRESS: (*Summarize progress relative to project goals and objectives. Highlight outstanding accomplishments, outreach and education efforts; describe problems encountered and explain any delays.)*

Summary

We have installed equipment and collected extensive hydrologic and chemical data at five sites (Davis, Thornton, Lois, Marion, and Elderslie). We have also measured plant cover and diversity and soil carbon and nitrogen at each site. Statistical analysis has begun.

Davis

The Davis stormwater treatment system, located near the corner of Davis St. and Hartford Turnpike in Hamden, CT, was installed by the South Central Connecticut Regional Water Authority (SCCRWA). It consists of 3 ponds: a small shallow forebay; a larger, deeper, open-water pond; and a wetland dominated by *Typha sp*. It drains a 24 acre watershed, comprised of 23% impervious surface. The inlet and outlet both consist of reinforced concrete pipes ending in a concrete flare.

Due to the flashiness of the site and the low water levels in the pipes during most events, we were initially unable to accurately measure flows. In spring 2011, we constructed and installed custom V-notch weirs at both the inlet and the outlet, and placed water level loggers in both weir pools. This allowed us to use the weir equation to convert flow to water level. We have also obtained flow measurements using the dye dilution method[1], which have generally corroborated the use of the weir equation at moderate flows. We still need to obtain dye dilution measurements at high flows, since this part of our rating curve[2] is both critical and more poorly defined (as water completely fills the notch at flows above ~30 L/sec). Nonetheless, we are reasonably confident in the flow estimates shown below.

To date, we have sampled at Davis for a total of approximately 19 months, divided into three periods: 6/15/2011-12/15/2011; 4/11/2012-12/11/2012; and 4/12/2013-10/9/2013. During this time, we have a continuous record (5 minute interval) of water levels (and thus flow) at both the inlet and the outlet. In addition, we collected water samples over the course of 92 storms (Figs. 1 and 2). We also periodically collected samples from the pond in between storms, as well as quality control (QC) samples, for a total of 407 water samples from Davis (Table 1).

[1] Dye dilution involves adding a precisely-measured, very small amount of a fluorescent dye upstream of the location where flow is to be estimated. Downstream measurements of dye concentration are then used to calculate water flow rates based on the conservation of mass. Under certain circumstances, the same dye injection can be used to estimate residence time (see below).
[2] A rating curve is the mathematical relationship between water level and flow.

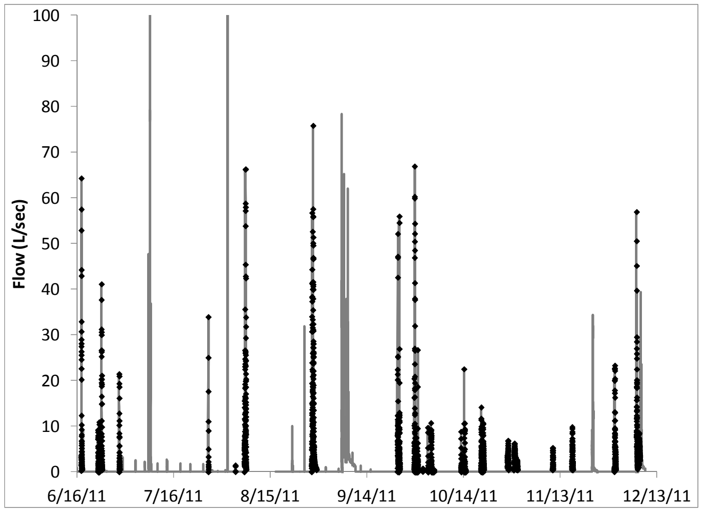


Figure 1. Flow at Davis In during time period 1. Black symbols indicate storms for which samples were collected.

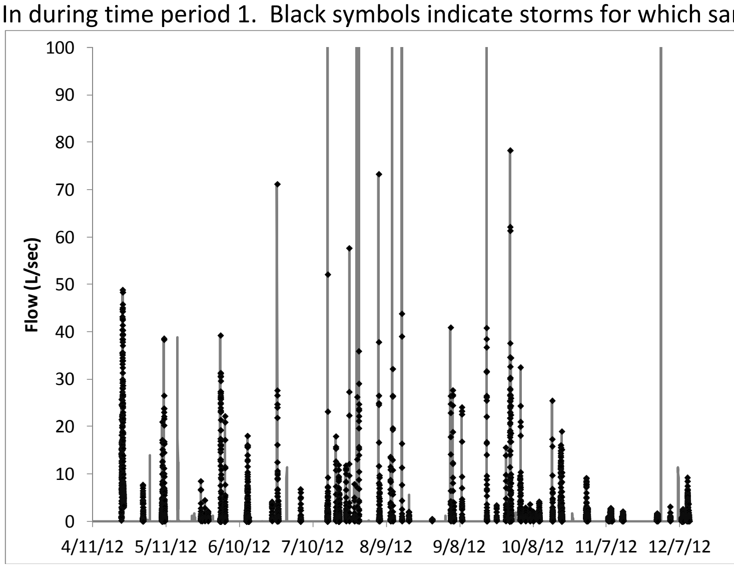


Figure 2. Flow at Davis In during time period 2. Black symbols indicate storms for which samples were collected.

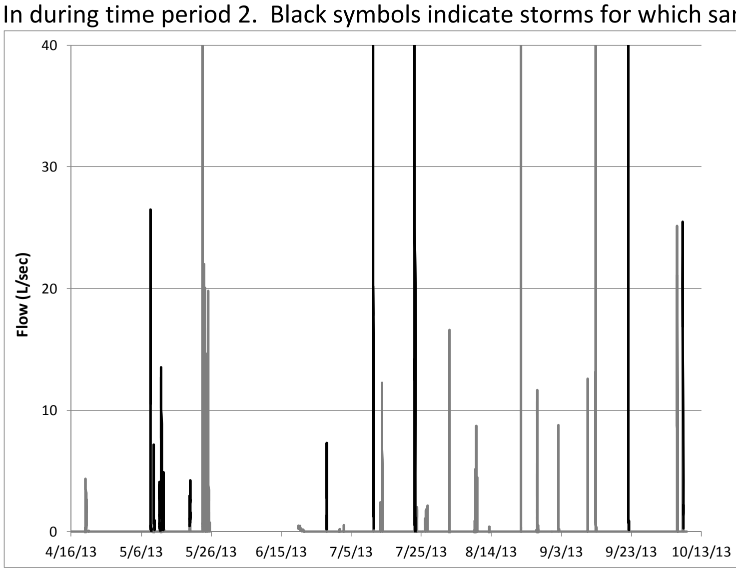


Figure 3. Flow at Davis In during time period 3. Black lines indicate storms for which samples were collected.

Thornton

The Thornton stormwater treatment system, located near the corner of Thornton St. and Greenway St. in Hamden, CT, was also installed by the SCCRWA. It consists of a small shallow forebay and a slightly larger wetland. It drains a 27 acre watershed with a curve number of 91. The inlet and outlet both consist of plastic pipes ending in plastic attachments.

We initially believed that we could measure flow at this site without installing weirs. We collected several storms' worth of data before discovering that during extended flow events, the forebay water level rose enough that it backed up into the inlet pipe. As a result, we installed custom-built Cipolletti weirs at both the inlet and the outlet in August-September 2012. We have been using the weir equation to calculate flows (from water level data), and have obtained dye dilution flow data to corroborate this.

To date, we have sampled at Thornton for approximately 7 months, collecting samples during 40 storms, for a total of 76 samples (Figure 4 and Table 1).

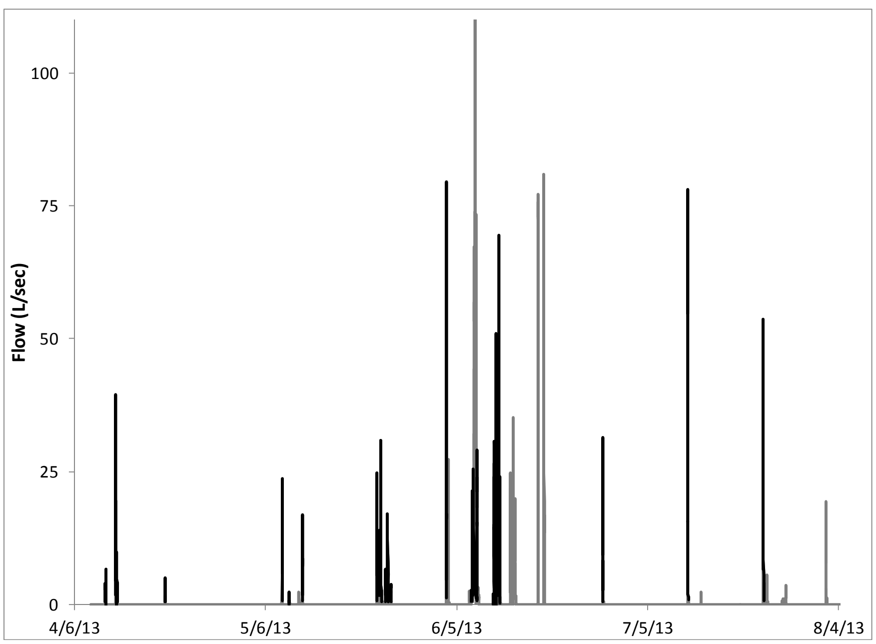


Figure 4. Flow at Thornton In during 2013. Black lines indicate storms for which samples were collected.

Table 1. Number of storms sampled and number of samples collected. Note that some smaller storms produced only inflow and no outflow.

Site	Time Period	Number of storms	Number of samples
Davis In	June-Dec 2011	23	60
Davis Out	June-Dec 2011	18	42
Davis QC and dry period	June-Dec 2011	NA	57
Davis In	April-Dec 2012	57	112
Davis Out	April-Dec 2012	41	73
Davis QC and dry period	April-Dec 2012	NA	25
Davis In	April-Oct 2013	12	20
Davis Out	April-Oct 2013	9	10
Davis QC and dry period	April-Oct 2013	NA	8
Davis Total	**June 2011 – Oct 2013**	**92**	**407**
Thornton In	Sep – Dec 2012	17	24
Thornton Out	Sep – Dec 2012	7	11
Thornton In	Apr-Aug 2013	23	23
Thorton Out	Apr-Aug 2013	18	18
Thornton Total[3]	**Sep 2012 – Aug 2013**	**40**	**76**
Lois In and Out	Aug 2011 – June 2012	7	130[4]
Lois In	June-Nov 2013	11	13
Lois Out	June-Nov 2013	9	9
Lois Total	**Aug 2011 – Nov 2013**	**18**	**151**
Marion In	Aug-Dec 2013	9	9
Marion Out	Aug-Dec 2013	9	9
Marion Total	**Aug-Dec 2013**	**9**	**18**
Elderslie In	Nov-Dec 2013	4	6
Elderslie Out	Nov-Dec 2013	1	1
Elderslie Total	**Nov-Dec 2013**	**4**	**7**
Total		**163**	**659**

Lois

The Lois stormwater treatment system, located on Lois Drive, Woodbridge, CT, was installed by the town of Woodbridge. It consists of a small forebay and a wetland basin with a mixture of herbaceous and woody vegetation. The inlet is a plastic pipe which is partially submerged from the water in the forebay and thus cannot be used to measure inflow. The outlet is a concrete

[3] This does not include 46 samples collected before installation of the weirs.
[4] The high number of samples per storm at Lois is due to the fact that we did not composite samples at this site; see below.

control structure with 6 orifices of different sizes and placements. Outflow moves through the orifices into a chamber and then out through a reinforced concrete pipe.

To measure flows at the outlet, we used a water level logger in the outlet pipe, together with the Manning equation. These measurements are corroborated with a second water level logger at the control structure, where we use orifice equations to convert level to flow.

Flows at the inlet have been harder to measure. After some exploration, we installed a water level logger inside a manhole upstream of the inlet pipe. However, the difficulty of accessing the logger caused us to switch to a different kind of logger, which allows us to access the data without entering the manhole. For 13 storms, we used the Manning equation for converting level to flow. In October 2013, we installed a custom-made weir in the inlet, and used the weir equation for the final 5 storms.

We have collected a total of 151 samples over 18 storms at Lois (Table 1 and Figure 5).

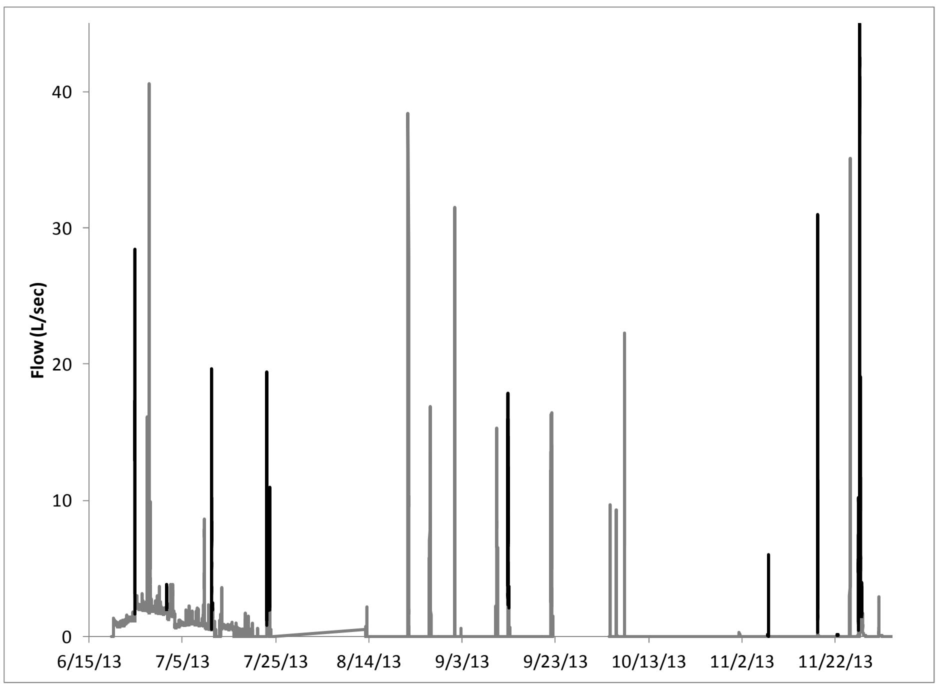


Figure 5. Flow at Lois In during 2013. Black lines indicate storms for which samples were collected.

Marion

The Marion stormwater treatment system, located on Marion Drive, Woodbridge, CT, was installed by the town of Woodbridge. It consists of a small forebay and a wetland basin with a mixture of herbaceous and woody vegetation. There appears to be short-circuiting of the flow pathway, as the inlet and outlet are adjacent to each other, and much of the flow appears not to enter the larger part of the system.

The inlet is a reinforced concrete pipe with shallow slope; flows were measured using a water level logger and the Manning equation. The outlet is a concrete control structure with 6 orifices of different sizes and placements; flows were measured using a water level logger and the orifice equation.

We collected 18 samples over 9 storms at Marion (Table 1 and Figure 6).

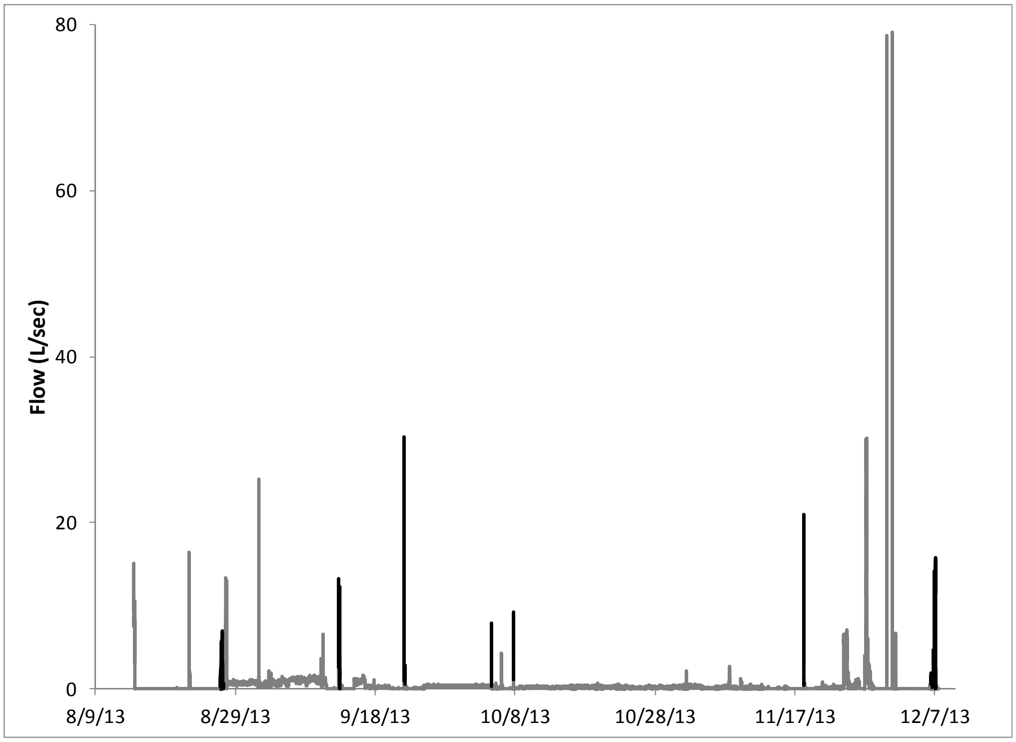


Figure 6. Flow at Marion In during 2013. Black lines indicate storms for which samples were collected.

Elderslie

The Elderslie stormwater treatment system, located on Elderslie Lane, Woodbridge, CT, was installed by the town of Woodbridge. It consists of a small forebay and a large wetland basin with a mixture of herbaceous and woody vegetation.

The inlet is a plastic pipe which is partially submerged from the water in the forebay and thus cannot be used to measure inflow. We installed a customized weir in a manhole upstream from the inlet, and used a water level logger and the weir equation to calculate flow. The outlet is a concrete control structure with 4 orifices of different sizes and placements; we used a water level logger and the orifice equation to calculate flow.

We sampled 4 storms from this site, only one of which had outflow as well as inflow (Table 1).

Sampling

At each of our sites, sample collection and processing involved five steps:

1. autosampler (AS) sample collection: Autosamplers were programmed to begin sampling upon an increase in water level (detected with a water-sensitive trigger) and to collect sub-samples into pre-cleaned glass bottles every 6-12 minutes throughout the storm event. Generally, 5 sub-samples were collected into each bottle, so that each AS sample represented a 30-60 minute period.
2. compositing: In order to maximize the accuracy of our nitrogen flux estimates while keeping the number of samples reasonable, we manually created composite samples for each storm, by mixing AS samples in the lab in volumes that were proportional to the volume of flow that they represented. We generally created 1-2 composite samples for each pipe (in/out) for each storm, although the samples representing high flows (>25 L/sec) were not composited but rather processed individually. At Lois, we did not composite any of our samples from 2012, because we were not confident in our flow estimates at the time of sample collection.
3. processing: Samples (composite or individual) were filtered through pre-washed, pre-weighed filters in order to determine TSS (total suspended solids). Both filtered and raw samples were immediately placed in the freezer for later analysis.
4. sample analysis: Samples were thawed completely and analyzed for concentrations of: NO_3^-, SO_4^{2-}, and Cl^- (ion chromatography); total N and total P (alkaline persulfate digestion followed by flow analysis); and dissolved organic carbon (TOC analyzer).
5. flux calculation: For each storm, we calculate the N fluxes in and out of the pond by multiplying flows by the corresponding concentrations, as illustrated in Figure 7 for a sample storm. The wetland was judged to be removing N when fluxes out were substantially less than fluxes in. This can occur for a combination of two reasons: less water is flowing out than in (because of storage and infiltration); and the outflowing water is lower in N than the inflowing water. Both factors are at play in the example shown.

Site Characterization

In addition to obtaining basic data on wetland and watershed size, we sampled each wetland in fall 2013 for plant species composition and cover. We also collected soil samples, which were dried and measured for C and N content.

Data analysis

We have compiled our master spreadsheets and calculated fluxes for individual storms. We have begun statistical analysis to understand the factors affecting outflow concentrations and loads. Fuller details and results will be in our final report.

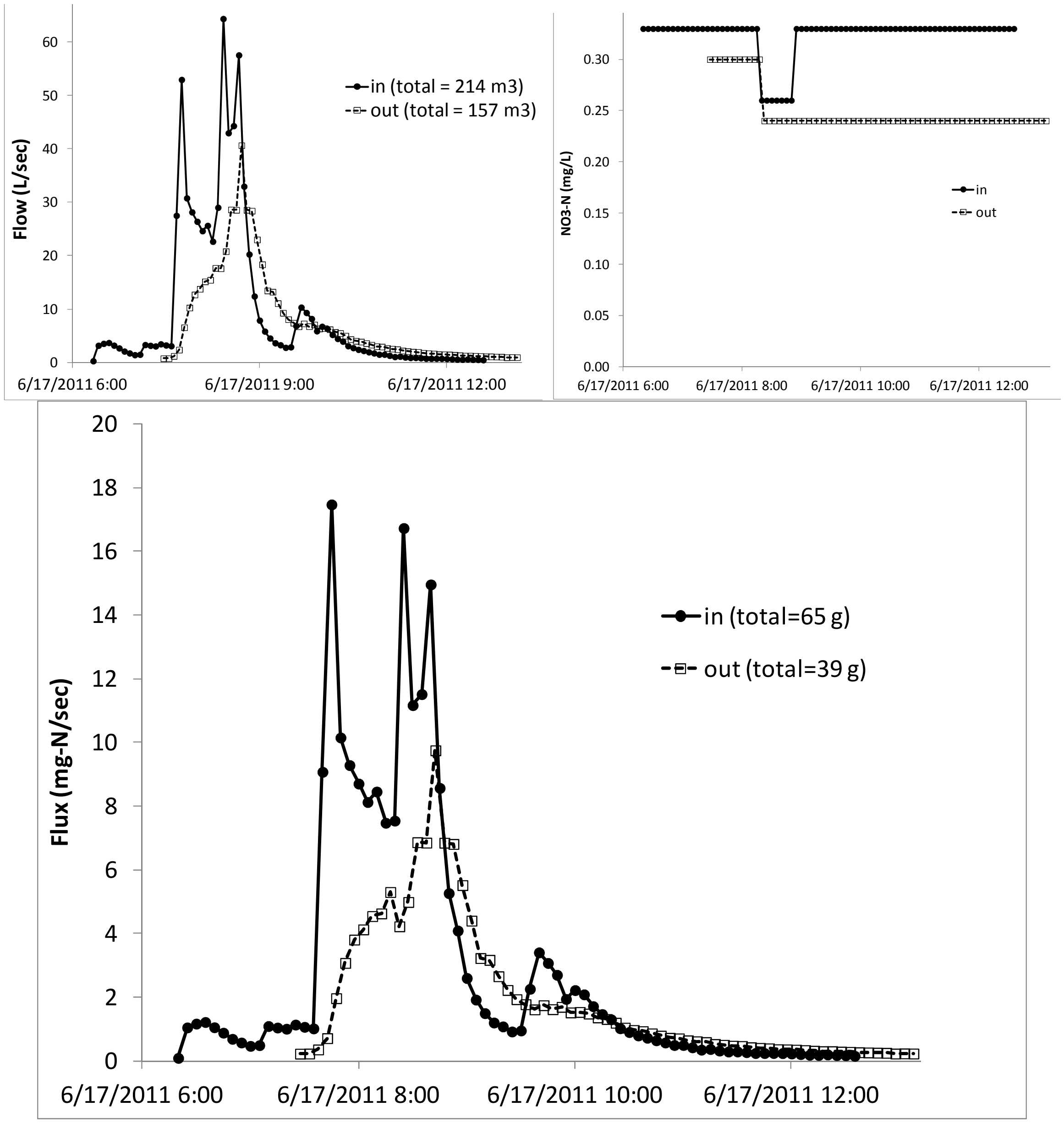


Figure 7. Top left: Flow of water in and out of Davis for a sample storm. Top right: Concentration of NO_3^- in inflow and outflow samples (n=2 each) for the same storm.[5] Bottom: Calculated flux of NO_3^- in and out of Davis for the same storm. Wetland N removal for this storm is ~40% (26g out of 65 g).

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[5] Note that each of these samples is a weighted composite, so it captures more of the temporal variability than appears in the graph.

Problems Encountered

The most significant problem that we have encountered has been the difficulty of instrumenting sites to adequately measure flows. Accurate and precise hydrologic measurements are critical to achieving our objectives, and we have put a great deal of effort into this aspect of the work. At this point, after much trial and error, we have successfully installed 6 custom-built weirs.

An additional aspect of our hydrologic difficulties has been the challenge of corroborating the rating curves that we have been using. While weir rating curves are generally quite good, we feel that it is necessary to ensure that they apply to our sites, especially at the upper end of flows at Davis, when the V-notch is full and the weir essentially becomes a rectangular weir. We have been using a low-cost ADCP instrument (Starflow) to obtain continuous unattended velocities, but this has proven unreliable. Instead, we have turned to dye dilution flow measurements. Again, as with the weir installation, there has been a steep learning curve for this technology (mostly having to do with obtaining precise pumping rates in the field), but we are now at a point where we have the method worked out. The challenge that still remains is to capture the flows manually during these relatively rare high flow events, which tend to be unpredictable and at inconvenient times. Part of the challenge is the flashiness of these sites, which experience peak flows (at the inlet) within minutes of a burst of rain.[6]

D. <u>PROJECT PUBLICATIONS, PRODUCTS AND PATENTS</u>: *(Include published materials with complete references, as well as those which have been submitted but not yet published and those in press. Please attach electronic versions of any journal articles not previously provided.)*

Journal Articles:

Conference Papers and Presentations:

1. **Lisa Weber, American Museum of Natural History's Student Conference on Conservation Science, New York, NY** (October 10-13, 2012), "Reducing Hypoxia Levels in Long Island Sound with Connecticut Constructed Wetlands"
2. **Lisa Weber, Yale University's Hixon Center for Urban Ecology Fellow Presentation, New Haven, CT** (March 4, 2013), "Inter-storm variability in nitrogen removal in a Connecticut constructed wetland"
3. **Lisa Weber, Connecticut Association of Wetland Scientists: 2013 Annual Meeting, Southbury, CT** (March 21, 2013), "Examining the Efficacy of Connecticut Constructed Wetlands as a Stormwater Best Management Practice"

[6] We have had several almost-successful attempts, where a very promising storm has fizzled after we were ready and set up.

4. **Lisa Weber, Yale University's Master of Environmental Science Colloquium, New Haven, CT** (April 19, 2013), "Inter-storm variability in nitrogen removal in a Connecticut constructed wetland"

5. **Zulimar Lucena, Conference on green infrastructure and water management in growing metropolitan areas, Tampa, FL** (January 14, 2014), "Factors controlling biogeochemical removal of nitrogen in constructed wetlands"

6. **Zulimar Lucena, Yale University's Hixon Center for Urban Ecology Fellow Presentation, New Haven, CT** (February 19, 2014), "Factors controlling biogeochemical removal of nitrogen in constructed wetlands"

7. **Zulimar Lucena, Connecticut Association of Wetland Scientists: 2014 Annual Meeting, Southbury, CT** (March 20, 2014), "Factors controlling biogeochemical removal of nitrogen in constructed wetlands"

Other articles, such as proceedings or book chapters:

Web sites, Software, etc.:

Technical Reports / Other Publications:

Other Products (including popular articles):

Planned Publications:

1. **Inter-storm variability in nitrogen removal in a Connecticut constructed wetland,** Lisa Weber, Shimon C. Anisfeld, Gaboury Benoit, and Zulimar Lucena

2. **Inter-site variability in nitrogen removal efficiency in constructed wetlands,** Zulimar Lucena, Shimon C. Anisfeld, Gaboury Benoit, and Lisa Weber

Patents: *(List those awarded or pending as a result of this project.)*

E. <u>FUNDS LEVERAGED</u>: *(If this Sea Grant funding facilitated the leveraging of additional funding for this or a related project, note the amount and source below.)*

Carpenter-Sperry Fellowship (Yale FES) awarded to Lisa Weber: **$2500**
Hixon Center Fellowship (Yale FES) awarded to Lisa Weber: **$7000**
Michael Lefor Grant (CT Association of Wetland Scientists) awarded to Lisa Weber: **$1000**
Hixon Center Fellowship (Yale FES) awarded to Zulimar Lucena: **$7000**
Michael Lefor Grant (CT Association of Wetland Scientists) awarded to Zulimar Lucena: **$1000**
Students paid from other accounts to work on this project (1290 hours): **$19,354**

F. **<u>STUDENTS</u>:** ***(****Document the number and type of students supported by this project.)*
*Note: **"Supported"** means supported by Sea Grant through financial or other means, such as Sea Grant federal, match, state and other leveraged funds. If a student volunteered time on this project, please note the number of volunteer hours below.*

Total number of **<u>new*</u>** K-12 students who worked with you:
Total number of **<u>new</u>** undergraduates who worked with you:
Total number of **<u>new</u>** Masters degree candidates who worked with you: 2
Total number of **<u>new</u>** Ph.D. candidates who worked with you:

Total number of **<u>continuing**</u>** K-12 students who worked with you:
Total number of **<u>continuing</u>** undergraduates who worked with you:
Total number of **<u>continuing</u>** Masters degree candidates who worked with you: 10
Total number of **<u>continuing</u>** Ph.D. candidates who worked with you:

Total number of volunteer hours:

*(Note: *__New__ students are those who have <u>not</u> worked on this project previously.*
*****__Continuing__ students are those who have worked on this project previously.)*

In the case of graduate students, please list student names, degree pursued, and thesis or dissertation titles related to this project.

new students:

Student Name: Michelle Camp
Degree Sought: MEM
Thesis or Dissertation Title:
Date of thesis <u>completion:</u>
Expected date of graduation: May 2015

Student Name: Kris van Naerssen
Degree Sought: MEM
Thesis or Dissertation Title:
Date of thesis <u>completion:</u>
Expected date of graduation: May 2015

continuing and graduated students:

Student Name: Zulimar Lucena
Degree Sought: MESc
Thesis or Dissertation Title: **Inter-site variability in nitrogen removal efficiency in constructed wetlands**

Date of thesis completion:
Expected date of graduation: May 2014

Student Name: Lisa Weber
Degree Sought: MESc
Thesis or Dissertation Title: **Inter-storm variation in nitrogen removal in constructed wetlands**
Date of thesis completion: May 2013
Expected date of graduation: May 2013

Student Name: Kevin Sherrill
Degree Sought: MESc
Thesis or Dissertation Title:
Date of thesis completion:
Expected date of graduation: May 2014

Student Name: Tatiana Hayek
Degree Sought: MEM
Thesis or Dissertation Title:
Date of thesis completion:
Expected date of graduation: May 2014

Student Name: Jocelyn Mahone
Degree Sought: MF
Thesis or Dissertation Title:
Date of thesis completion:
Expected date of graduation: May 2013

Student Name: Sarah Barbo
Degree Sought: MEM
Thesis or Dissertation Title:
Date of thesis completion:
Expected date of graduation: May 2013

Student Name: Bunyod Holmatov
Degree Sought: MEM
Thesis or Dissertation Title:
Date of thesis completion:
Expected date of graduation: May 2013

Student Name: Ariel Patashnik
Degree Sought: MEM
Thesis or Dissertation Title:
Date of thesis completion:

Expected date of graduation: May 2012

Student Name: Alison Schaffer
Degree Sought: MEM
Thesis or Dissertation Title:
Date of thesis completion:
Expected date of graduation: May 2012

Student Name: Kavita Sharma
Degree Sought: MEM
Thesis or Dissertation Title:
Date of thesis completion:
Expected date of graduation: September 2012

CONNECTICUT SEA GRANT PROJECT REPORT

Please complete this progress or final report form and return by the date indicated in the emailed progress report request from the Connecticut Sea Grant College Program. Fill in the requested information using your word processor (i.e., Microsoft Word), and e-mail the completed form to Dr. Syma Ebbin syma.ebbin@uconn.edu, Research Coordinator, Connecticut Sea Grant College Program. Do NOT mail or fax hard copies. Please try to address the specific sections below. If applicable, you can attach files of electronic publications when you return the form. If you have questions, please call Syma Ebbin at (860) 405-9278.

Please fill out all of the following that apply to your specific research or development project. Pay particular attention to goals, accomplishments, benefits, impacts and publications, where applicable.

Project #: **R/CE-32-CTNY** Check one: [ ] Progress Report [x] Final report

Duration (dates) of entire project, including extensions: From [3/1/2011] to [2/28/2013].

Project Title or Topic: Comparative Analysis of Eutrophic Condition and Habitat Status in Connecticut and New York Embayments of Long Island Sound.

Principal Investigator(s) and Affiliation(s):

1. Jamie Vaudrey, University of Connecticut

2. Charles Yarish, University of Connecticut

3.

4.

A. COLLABORATORS AND PARTNERS: *(List any additional organizations or partners involved in the project.)*
Not applicable.

B. PROJECT GOALS AND OBJECTIVES:

For the majority of the more than 60 embayments of LIS, very little is known about their current eutrophic condition, dominant habitat type, potential to support submerged aquatic vegetation (SAV), or changes in community structure due to climate change effects. Small coastal embayments are the receiving waters for much of the nonpoint source nitrogen (N) being delivered into LIS. This nitrogen input has been identified as a major contributing factor to eutrophication and the loss of SAV in estuaries. By sampling in many sites across a range of nitrogen loads, the relationship between stressors (N, temperature, flushing time) and estuarine response (hypoxia, macroalgae blooms, loss of SAV) can be investigated. This data set can also be used to examine sites for the potential to support SAV and serve as a baseline for future work related to eutrophication issues and climate change effects.

Objectives:

1. Survey the habitat characteristics in 8 embayments of Long Island Sound.

2. Utilize standard indicators of estuarine water quality and eutrophication status (EPA, NOAA) to assess the relative "health" of these estuaries, comparing within Long Island Sound and to estuaries throughout the nation.

3. Assess these sites for their potential to support *Zostera marina* L. (eelgrass).

4. Identify the links between stresses to the embayments (nitrogen load, temperature), contributing factors (freshwater flushing time, size), and estuarine response (indices from #2, habitat characteristics).

5. Compare the habitat characteristics to historic data, where available. This includes evaluating changes in temperature and pH as possible indicators of climate change effects.

6. Develop a baseline set of data for future researchers working in Long Island Sound.

7. Present results to the scientific community, Long Island Sound managers, and stakeholders.

8. Introduce 6 undergraduate students (3 / y) to ecological field research, lab techniques, and data analysis for use in informing management and scientists on ecosystem status of small embayments.

C. PROGRESS: (*Summarize progress relative to project goals and objectives. Highlight outstanding accomplishments, outreach and education efforts; describe problems encountered and explain any delays.)*

1. All 8 sites were sampled once during the predicted height of hypoxia (end of July & early August) in 2011 and 2012. Data have been synthesized into a summary file, linked to individual data files.
2. Field data were used to compare the embayments for eutrophic status using the NOAA ASSETS model (http://eutro.org/, http://www.eutro.org/register/). This approach includes an estimate of nitrogen loading, flushing time, and information on key indicators (primary symptoms: chlorophyll *a*, macroalgae; secondary symptoms: dissolved oxygen, nuisance and toxic algae blooms, submerged aquatic vegetation).
3. Sites were assessed for *Zostera marina* suitability using a GIS based site suitability model, completed as part of a LISS/NEIWPCC funded project (Vaudrey and Yarish are PIs on this project). Data from this project were used to enhance the data input for the GIS model. The final report for the NEIWPCC project, including presentation of the model results, will be posted at the UConn Digital Commons site (http://digitalcommons.uconn.edu/). The report has been submitted and will be released on the site shortly (once all authors send in acceptance of the submission agreement).
4. Links between stresses to the embayments (nitrogen load, temperature), contributing factors (freshwater flushing time, size), and estuarine response (indices from #2, habitat characteristics) were identified. A summary of findings is provided in the results section below.
5. Habitat characteristics were compared with historic data, where possible. In many sites, a comparable data set (similar time of year, similar techniques) were not available.
6. A baseline set of data was developed and will be available for future researchers working in Long Island Sound. The data will be made available through the UConn Digital Commons, upon completion of the peer-reviewed journal articles which will utilize the data. Data are available

upon request. For example, a Ph.D. student working with Dr. Melanie Fewings at UConn is using the temperature data form deployed sensors to estimate heat budgets in Long Island Sound embayments.

7. Present results. A list of presentations is included in section D of this report.
8. Six students were funded fully or partially by this project during the summer of 2011 and five students worked on the project during the summer of 2012. Two to five students worked part time during the school year throughout the project.

D. <u>PROJECT PUBLICATIONS, PRODUCTS AND PATENTS</u>: *(Include published materials with complete references, as well as those which have been submitted but not yet published and those in press. Please attach electronic versions of any journal articles not previously provided.)*

Journal Articles:

Conference Papers and Presentations:

Vaudrey, J.M.P. (2014) *Eutrophic condition and habitat status in Connecticut and New York embayments of Long Island Sound.* invited presentation, Southern Connecticut State University.

Vaudrey, J.M.P. (2014) *The Breathing of the Bays: a journey into green water.* invited presentation, Faulkners Light Brigade Lecture Series, Guilford.

Vaudrey, J.M.P. (2013) *Using Nitrogen Budgets as a Tool to More Effectively Manage Long Island Sound Embayments*. 2nd Workshop on Using Cultivated Seaweed and Shellfish for Nutrient Bioextraction in LIS and the Bronx River Estuary, Mamaroneck, NY.

Vaudrey, J.M.P. (2013) *Marine Ecosystem Ecology.* invited presentation at the “Women In Science” program for middle school girls, The Sound School.

Vaudrey, J.M.P. and C. Yarish (2013) *Nitrogen loading to embayments of Long Island Sound: method review and potential utility to management*. Presentation to the Long Island Funders Collaborative Meeting, New York City, NY. 01 Mar 2013.

Vaudrey, J.M.P. and C. Yarish (2012) C*omparative Analysis of Eutrophic Condition and Habitat Status in Connecticut and New York Embayments of Long Island Sound*. Presentation to the LISS STAC, 16 Nov 2012.

Yarish, C. and J. Vaudrey (2011) C*omparative Analysis of Eutrophic Condition and Habitat Status in Connecticut and New York Embayments of Long Island Sound*. Presentation to the LISS STAC, 18 Nov 2011.

Other articles, such as proceedings or book chapters:

Web sites, Software, etc.:

Technical Reports / Other Publications:

Other Products (including popular articles):

Zaretsky, M. (8/4/13) *Low oxygen levels present even in bays in eastern estuary, UConn researchers find.* interview, article, and photos for the New Haven Register. (http://www.nhregister.com/general-news/20130804/low-oxygen-levels-present-even-in-bays-in-eastern-estuary-uconn-researchers-find; http://photos.newhavenregister.com/2013/08/01/photos-marine-researcher-studies-l-i-sound-oxygen/#1)

Vaudrey, J.M.P. (2012) *The Breathing of the Bays*. Wrack Lines (Spring/Summer 2012): 5-7.

Vaudrey, J. and C. Yarish (2012) *Taking the Pulse of Long Island Sound's Embayments*. Short article submitted to the 2012 Sound Health Indicators report for the Long Island Sound Study.

Planned Publications:

Vaudrey, J.M.P., A. Chlus, A. Branco, C. Yarish, J. Kremer (in prep) Nitrogen inputs to Long Island Sound embayments from the NLM (Nitrogen Loading Model): estimates vary with methods used for estimating population.

Vaudrey, J.M.P., J. Kim, C. Yarish (in prep) Macrophyte elemental composition indicates the degree of nitrogen loading to embayments.

-- evaluation of hypoxia in embayments relative to Long Island Sound

-- evaluation of hypoxia in embayments relative to stressors (N-load, flushing time, etc.)

Patents: *(List those awarded or pending as a result of this project.)*

E. **FUNDS LEVERAGED:** *(If this Sea Grant funding facilitated the leveraging of additional funding for this or a related project, note the amount and source below.)*

F. **STUDENTS:** ***(****Document the number and type of students supported by this project.)*
Note: ***"Supported"*** *means supported by Sea Grant through financial or other means, such as Sea Grant federal, match, state and other leveraged funds. If a student volunteered time on this project, please note the number of volunteer hours below.*

Total number of **new*** K-12 students who worked with you: 0
Total number of **new** undergraduates who worked with you: 6 in 2011; 4 in 2012
Total number of **new** Masters degree candidates who worked with you: 0
Total number of **new** Ph.D. candidates who worked with you: 0

Total number of **continuing**** K-12 students who worked with you: 0
Total number of **continuing** undergraduates who worked with you: 0 in 2011; 3 in 2012
Total number of **continuing** Masters degree candidates who worked with you: 0
Total number of **continuing** Ph.D. candidates who worked with you: 0

Total number of volunteer hours: 0

*(Note: *__New__ students are those who have <u>not</u> worked on this project previously. **__Continuing__ students are those who have worked on this project previously.)*

In the case of graduate students, please list student names, degree pursued, and thesis or dissertation titles related to this project.

Student Name:
Degree Sought:
Thesis or Dissertation Title:
Date of thesis <u>completion:</u>
Expected date of graduation:

G. **<u>PICTORIAL</u>:** Provide high resolution images/photos of personnel at work, in the field or laboratory, equipment being used, field sites, organism(s) of study. Attach images as separate files (do not embed). Include links to websites associated with the research project. Please include proper photo credits and a caption with date, location, names of people, and activity. These images are useful to document your project in future CTSG publications, websites and presentations.

see photos included with the report submitted in 2012

<u>FOR FINAL REPORTS ONLY, PLEASE COMPLETE THIS SECTION:</u>

H. PROJECT OUTCOMES AND IMPACTS

RELEVANCE OF PROJECT: *(Describe briefly the issue/problem / identified need(s) that led to this work.)*

This work originated from a need to assess the eutrophic status of embayments in Long Island Sound. Monitoring of the main stem of Long Island Sound is ongoing, frequent, spatially extensive, and data produced are of excellent scientific quality. Data from the embayments tends to be temporally and spatially inconsistent and methods are often not comparable as different organizations conduct the work.

RESPONSE: *(Describe briefly what key elements were undertaken to address the issue, problem or need, and who is/are the target audience(s) for the work.)*

We developed a relatively rapid assessment method to evaluate the eutrophic status of embayments. The goal was to inform the research and management community of Long Island Sound as to the extent and degree of eutrophic symptoms in Long Island Sound. In addition, many community groups and foundations in the Long Island Sound region have expressed an interest in the results.

RESULTS: *(Summarize findings and significant achievements in terms of the research and any related education or outreach component; cite benefits, applications, and uses stemming from this project, including those expected in the future. Include qualitative and quantitative results.)*

All 8 sites (Figure 1) were sampled once during the predicted height of hypoxia (end of July & early August) in 2011 and 2012. Data have been synthesized into a summary file, linked to individual data files. The field data were used to assign a value to the eutrophic status each embayment. The nitrogen loading based on land use was estimated for each embayment. Additional forcing factors such as temperature and restrictions to water flow within the embayments were characterized. **The overall goal was to develop a better understanding of the linkages between the forcing factors and the expression of eutrophication.**

Figure 1: Map of embayments samples as part of the project.

A. Assigning a value to the eutrophic status for each embayment.

Field data were used to compare the embayments for eutrophic status using the NOAA ASSETS model (http://eutro.org/, http://www.eutro.org/register/). This approach is based on an estimate of nitrogen loading, flushing time, and information on key indicators (primary symptoms: chlorophyll *a*, macroalgae; secondary symptoms: dissolved oxygen, nuisance and toxic algae blooms, submerged aquatic vegetation). As consistent information on nuisance and toxic algae blooms were not available in all sites, “unknown” was entered into the model for all sites.

The NOAA ASSETS model ideally takes data which reflects the annual average. Thus, results are not directly comparable to sites with year-round data but can be used to compare among the sites sampled only during the summer (Figure 2).

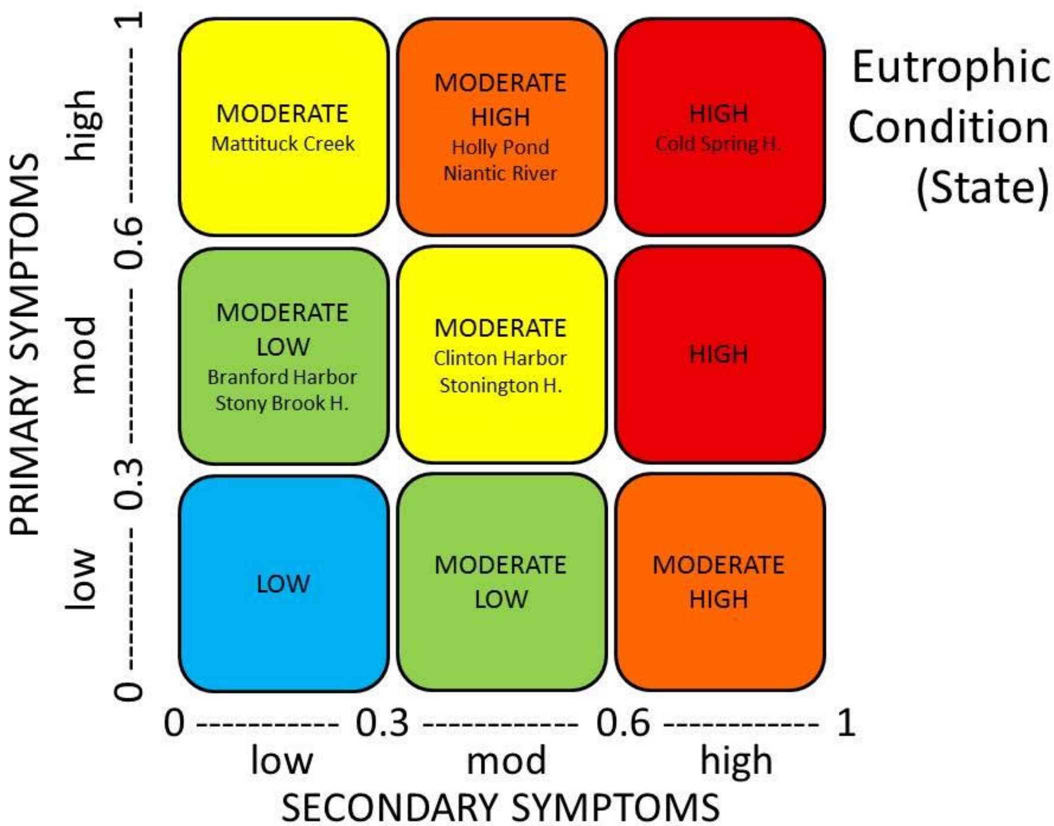

Figure 2: Eutrophic Condition as estimated by the NOAA ASSETS model, using site specific field data.

B. Estimating the nitrogen load to each embayment.

The nitrogen loads to the embayments were estimated using a land-use based model developed by Ivan Valiela[1] and modified by James Latimer and Michael Charpentier[2]. A paper is in draft form, detailing the model, comparing a variety of population estimates used for model input, and providing the N load for each of the embayments included in the study. The N load includes an estimate of nitrogen input based on land use category and population within the watershed. Sources were identified as wastewater inputs (septic and wastewater treatment facilities, WWTFs); fertilizer to homes, recreational fields, and agricultural fields; and atmospheric deposition to the watershed and directly to the surface of the embayment. Load estimates include both organic and inorganic forms of nitrogen and are the total delivered to the edge of the embayment. Load estimates include transformation and attenuation of N as it passes through the groundwater, but do not include any transformation or attenuation of N that occurs in saline water. The N load is presented as the total load to the embayment (Figure 3) and as the total load normalized to the area of the embayment receiving the nitrogen (Figure 4).

---

[1] Valiela, I., Collins, G., Kremer, J.N., Lajtha, K., Geist, M., Seely, B., Brawley, J., Sham, C.-H., 1997. Nitrogen loading from coastal watersheds to receiving estuaries: new method and application. Ecol. Appl. 7(2), 358-380.

[2] Latimer, J.S., Charpentier, M., 2010. Nitrogen inputs to seventy-four southern New England estuaries: application of a watershed nitrogen loading model. Estuar. Coast. Shelf Sci. 89, 125-136.

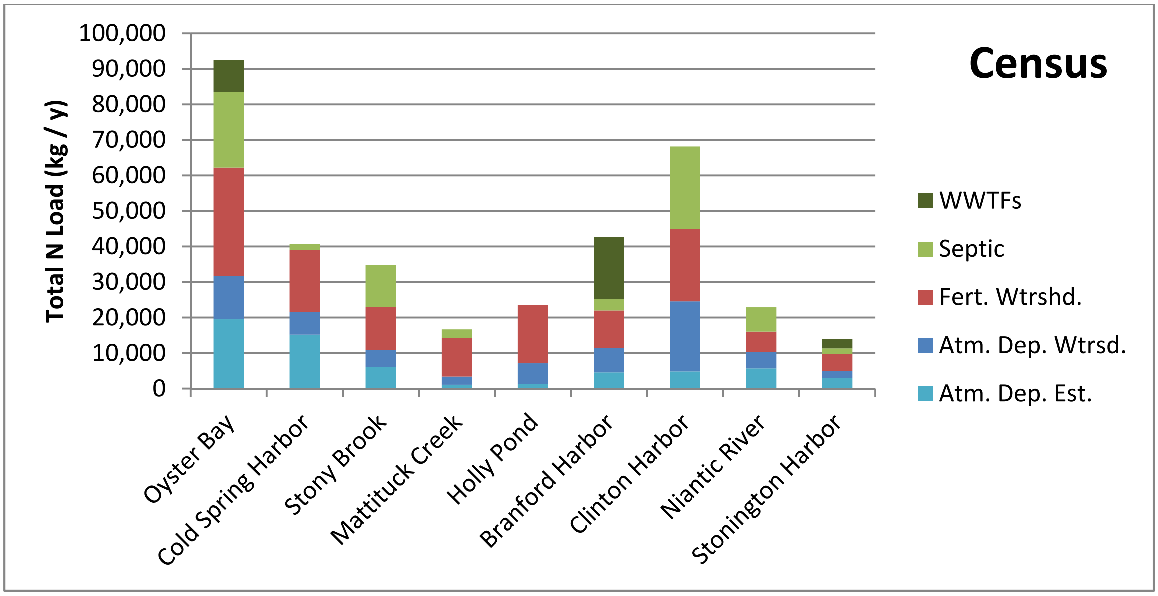

Figure 3: Results of Nitrogen Loading Estimates. Note that units are the total nitrogen load to the embayment.

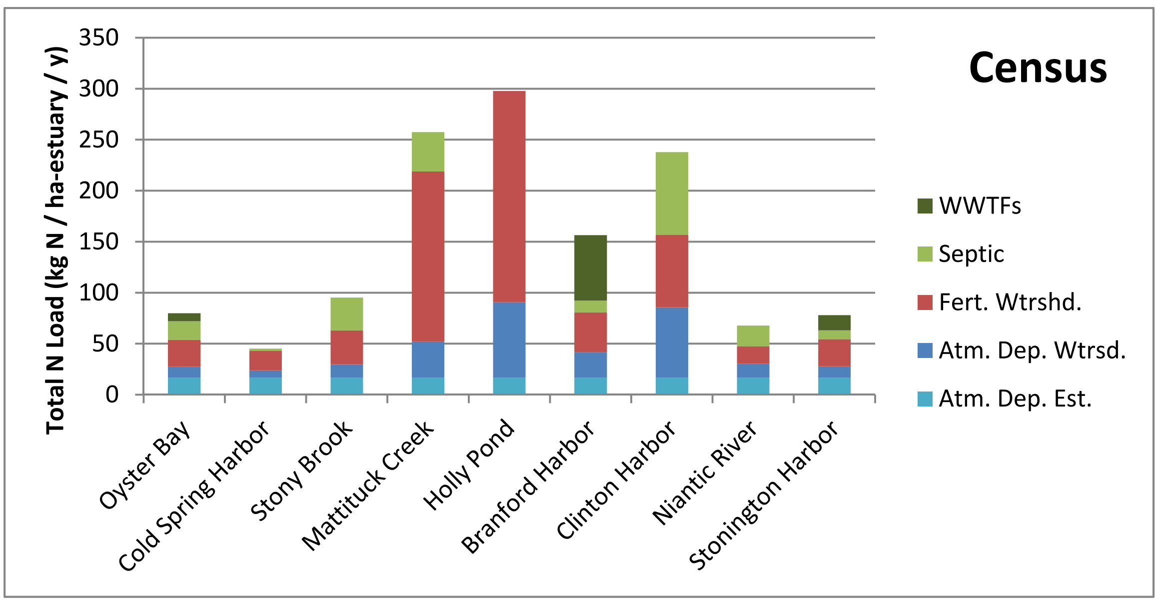

Figure 4: Results of Nitrogen Loading Estimates. Note that units are the total nitrogen load to the embayment normalized to the area of the embayment. Thus large loads to a large system (such as Oyster Bay) yield a lower per hectare$_{estuary}$ load while small loads to a very small area (such as Holly Pond) can yielda higher per hectare$_{estuary}$ load.

C. Investigating temperature as a factor influencing expression of eutrophic symptoms.

High temperatures in embayments have the potential to exacerbate the symptoms of eutrophication. In order to evaluate differences among sites and identify this possible effect, we deployed temperature sensors in the sites, at locations chosen based on the dawn sampling for hypoxia conducted in 2011. Most sensors remain deployed at this time and are downloaded every few months. The plan is to keep as many deployed as possible, to begin a long term record of temperature in these sites. A graduate student in the Department of Marine Sciences working with Dr. Melanie Fewings has proposed to use these data to examine the heat budget in shallow systems.

Some sensors have been lost, so gaps in the data are present. The Holly Pond sensor was lost without any data retrieved. This sensor was never redeployed due to the difficulty of deploying in this site (no docks present, sensor would have been exposed at low tide). The sensors in Stonington Harbor and Mattituck Creek were both lost due to dock movement and repairs, but both have been replaced. The sensor deployed in Cold Spring Harbor was exposed at low tide; only data for times when the sensor was deployed from a floating dock are presented.

The weekly trends in temperature are relatively consistent among sites, reflecting a commonality to weather and temperature throughout the Long Island Sound region, though some differences among sites are evident and expected (Figures 5 and **6**). In almost all sites, the temperature sensors were deployed at the head of the estuary, where hypoxia was most likely to occur. The exception was Stony Brook Harbor, where the sensor was deployed mid-way down the embayment. Niantic River and Mattituck Creek tend to be the two warmest stations (Figure **6**). Branford Harbor and Clinton Harbor are variable, occasionally exhibiting some of the highest temperatures and at other times, the coolest temperatures. Stonington Harbor is consistently cooler than other sites.

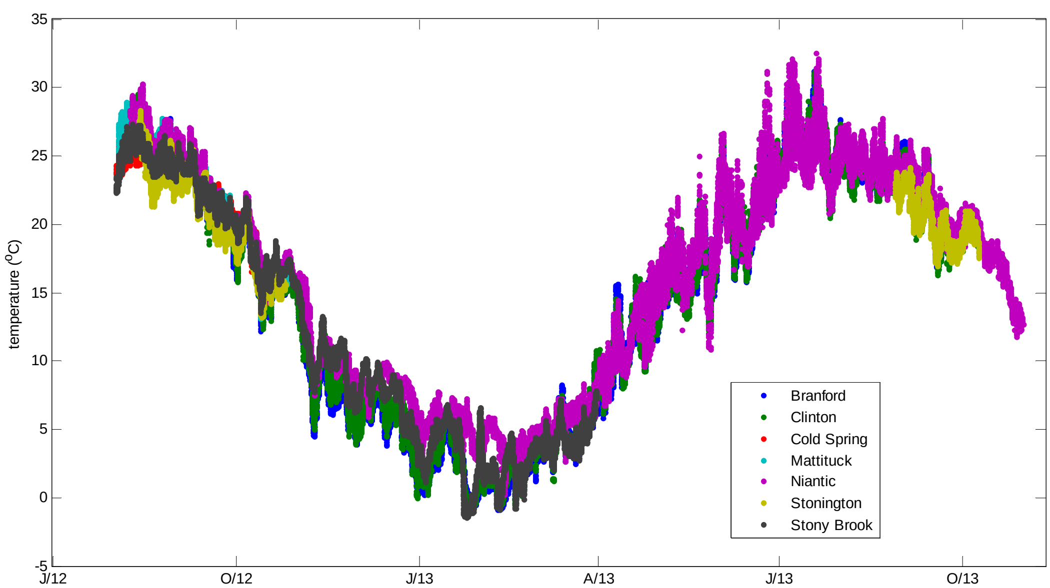

Figure 5: Temperature records from embayments. The sensor were deployed ~70 cm below the surface, suspended from a floating dock (actual depth of each sensor is available).

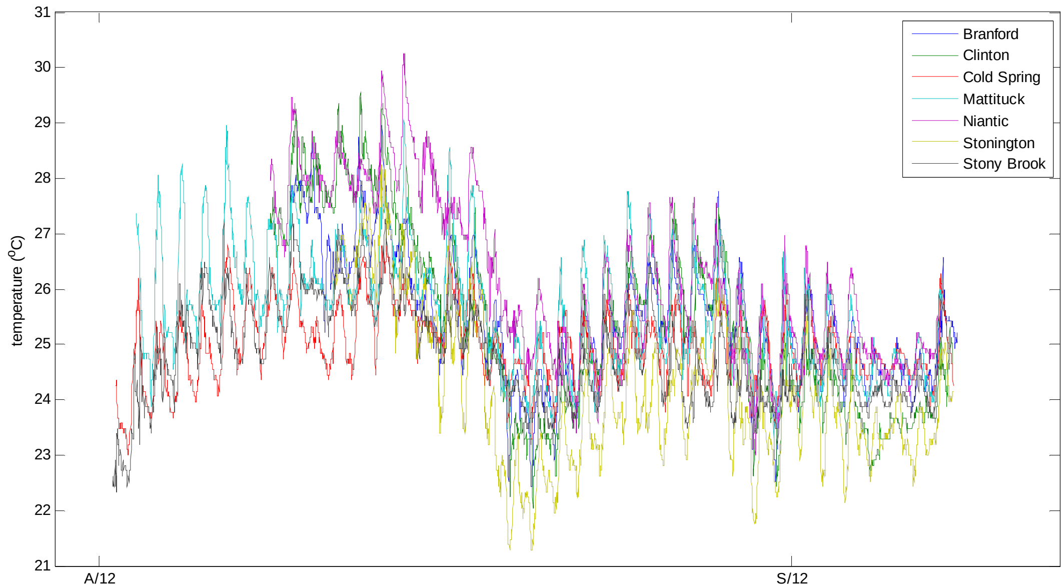

Figure 6: One month of temperature records from embayments, end of summer 2012. The figure shows a subset of the data presented in Figure 5. The sensors were deployed ~70 cm below the surface, suspended from a floating dock (actual depth of each sensor is available).

D. Key findings on the linkages between the forcing factors and the expression of eutrophication.

The estimate of eutrophic condition (Figure 2) correlates well with nitrogen load (Figure 4), if the flow restrictions in certain sites are taken into account. Cold Spring Harbor, Holly Pond, Niantic River, and Stonington Harbor all have some constriction restricting flushing of the embayment with long Island Sound water in some part of the embayment. Stonington has a train bridge separating the upper third of the estuary from the well-flushed southern portion. Niantic River has a train bridge at the southern end of the embayment and reduced flow in the upper fjord-like arm of the estuary, as well as a roadway bridge at the northern end further reducing flow to the northern tip of the embayment. Holly Pond has a dam at the southern limit of the embayment. Cold Spring Harbor has a sand bar separating the southern end of the embayment from the better flushed northern portion of the embayment.

For embayments with no flow restriction, the order from highest N load (kg N $ha_{estuary}^{-1}$ y^{-1}) to lowest is: Mattituck Creek, Clinton Harbor, Branford Harbor, Stony Brook. For embayments with a flow restriction which causes greater expression of eutrophic symptoms, the order from highest N load (kg N $ha_{estuary}^{-1}$ y^{-1}) to lowest is: Holly Pond, Stonington Harbor, Niantic River, and Cold Spring Harbor. For embayments with no flow restriction, the eutrophic condition is well-correlated with the trend in nitrogen loading. For those systems with a flow restriction, the correlation does not hold up as well. The temperature differences in these sites do assist with identifying eutrophic condition relative to N load. For example, Niantic River and Stonington Harbor have similar area normalized nitrogen loads (Figure 4), but the higher temperature in Niantic contributes to a eutrophic condition of “moderate high” while the cooler temperature of Stonington Harbor contributes to the “moderate” designation.

Early indication of these trends (following 2011) led to the development of a proposal to more thoroughly evaluate the nitrogen loading and eutrophic status in embayments of LIS, taking into account these flow restrictions. The proposal was funded and work commenced in summer of 2013.

Additional Results of Note:

While visiting the 8 embayments, Dr. Yarish collected macroalgae samples. As part of independent and self-supported efforts, Dr. Yarish and his colleagues(including Prof. Yun-Xiang MAO and R. Wilson) conducted DNA analysis of the samples to determine the presence of non-native species. In Holly Pond, the entire area has been overtaken by the invasive *Gracilaria vermiculophylla* (an invasive from the western Pacific). We have now confirmed that the dominant Gracilaria in the Mattituck Creek, Cold Spring Harbor and Stony Brook Harbor systems are the native cogenorer, *Gracilaria tikvahiae*. We also have some exciting results on some of the Ulvalean algae we have collected, but are waiting for Prof. Mao to decide how he wishes to publish that information.

During the summer of 2012, the massive amounts of algae washing up along the eastern shore of Stonington Borough (Ash Street beach) resulted in complaints from local residents. Vaudrey and Yarish (as well as marine scientists from Williams-Mystic, CT Sea Grant, and University of Rhode Island) consulted with town officials regarding the source of the problem. Our work in the area provided some context for the source of the problem – the massive blooms of macroalgae occurring in Little Narragansett Bay. The macroalgae bloom is fueled by nutrients entering the system, with the most likely source being the Pawcatuck River. A UConn undergraduate has applied for a UConn Summer Undergraduate Research Fellowship and a UConn IDEA grant to further identify the source of the nitrogen and characterize the extent of the macroalgae bloom. Her proposal developed out of her work on this grant and a desire to further explore this issue.

Consider the following as they apply to your research and any related outreach/education.

- What new tools, technologies, methods or information services were developed from this work? Have any been adopted / implemented for use and by whom?

  While not wholly new, we have demonstrated that a rapid assessment approach (a single day of sampling per site) can be used to compare the eutrophic status of embayments. Ideally, sampling of these systems would occur multiple times a year and carry on for multiple years. However, a day of sampling during the height of the hypoxic season provides information on the status of these systems. This single day of sampling proves useful because many of the indicators used integrate over a longer time period, for example, macroalgae persists for weeks to months in a system.

- What are the environmental benefits of this work? Have policies been changed? How has conservation (of ecosystems, habitats or species) been improved?

  The environmental benefits of this work include a much clearer and accurate understanding of the habitat characteristics in embayments and the dynamics of nutrient input and eutrophication.

- What are the social payoffs of this work? Who has benefited from this work? Have attitudes / behaviors of target audience changed? Elaborate. Have policies been changed?

  One benefit not highlighted in the results section is the identification of the sources of nitrogen in the embayment watersheds. For example, in the Cold Spring Harbor watershed, a large fraction of the estimated nitrogen load originates from fertilizer applied to recreational fields (Figure 7). If assumptions of the model are correct (amount of fertilizer applied, amount of area fertilized), a reduction of fertilizer to these fields would assist with the reduction of nutrients to this system. Results from this project will be made available to groups with interest in nutrient reductions, if such groups within a watershed can be identified. When groups within a watershed do not exist, these data will be provided to Save the Sound of CT Fund for the Environment in Connecticut and the Citizens Campaign Fund for the Environment in NY.

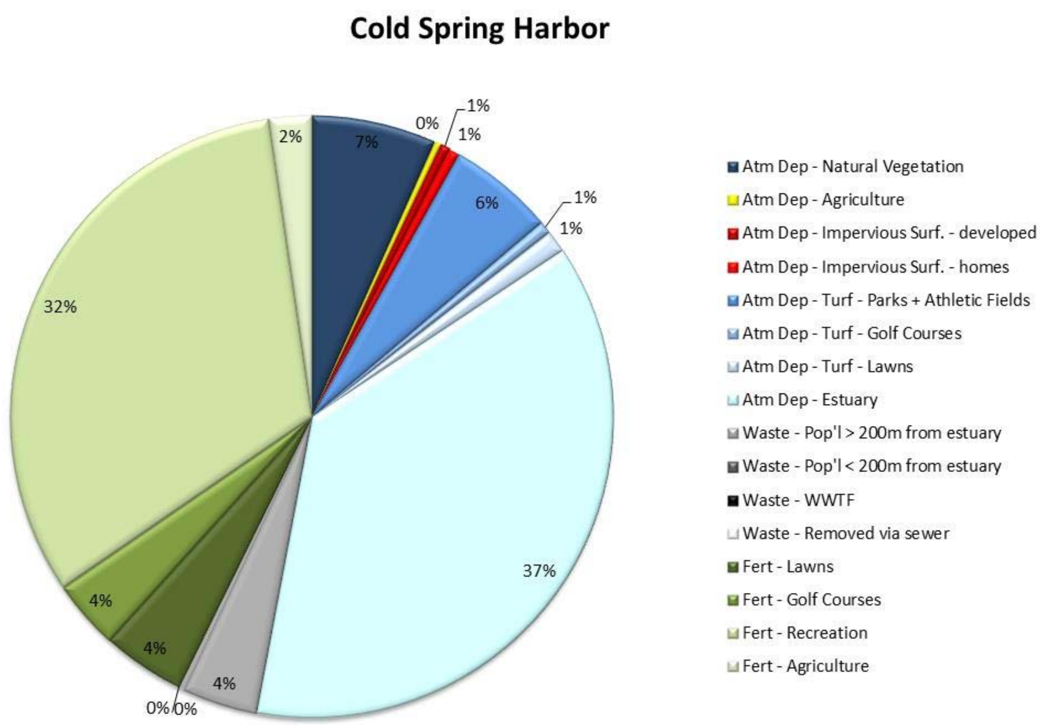

Figure 7: Sources of nitrogen delivered to Cold Spring Harbor

- What are the economic implications / impacts of this work? (Where possible, please quantify.) Have new businesses been created /or existing businesses retained as a result of this research? Have new jobs been created or retained? Are new businesses or jobs anticipated?

  No new jobs or businesses have been created as a result of this work. The economic implications bear upon the mandate for nutrient reductions entering Long Island Sound. Results from this work can be used to target systems most in need of mitigation efforts and also help to identify key sources of non-point source nitrogen within each watershed.

CONNECTICUT SEA GRANT PROJECT REPORT

Please complete this progress or final report form and return by the date indicated in the emailed progress report request from the Connecticut Sea Grant College Program. Fill in the requested information using your word processor (i.e., Microsoft Word), and e-mail the completed form to Dr. Syma Ebbin syma.ebbin@uconn.edu, Research Coordinator, Connecticut Sea Grant College Program. Do NOT mail or fax hard copies. Please try to address the specific sections below. If applicable, you can attach files of electronic publications when you return the form. If you have questions, please call Syma Ebbin at (860) 405-9278.

Please fill out all of the following that apply to your specific research or development project. Pay particular attention to goals, accomplishments, benefits, impacts and publications, where applicable.

Project #: _____________ Check one: [] Progress Report [X] Final report

Duration (dates) of entire project, including extensions: From [05/2012] to [12/2013].

a.) Project Title or Topic: Nitrogen Removal Capacity of Connecticut Estuaries: Assessing Distribution and Controls

Principal Investigator(s) and Affiliation(s):
1. Craig Tobias – University of Connecticut

2.

3.

4.

A. <u>COLLABORATORS AND PARTNERS</u>: *(List any additional organizations or partners involved in the project.)*

Bongkuen Song – Virginia Institute of Marine Science

B. PROJECT GOALS AND OBJECTIVES:

The overall goal of the project is to examine the rates and underlying factors that control dissolved inorganic nitrogen removal at representative river estuaries that discharge to Long Island Sound (LIS). We focus on both denitrification and ANAMMOX to examine potential controls on their rates resulting from changing microbial and geochemical factors. We concentrate efforts on river estuarine gradients rather than on the Sound itself for two reasons: 1) biogeochemical gradients found in the Sound (e.g. salinity, primary production, DIN concentration) are replicated in these estuaries on a compressed spatial scale thus rendering a more tractable examination of DIN removal over entire gradients; 2) working in the river estuaries targets our efforts nearest DIN sources entering the Sound via watershed discharge, where total DIN removal is most important on a system scale. Specific objectives are:

1) Quantify the N removal rates via denitrification and ANAMMOX in the Niantic river.

2) Determine how the overall N removal capacity changes throughout the estuary and map spatial and temporal N removal "hot-spots".
3) Quantify shifts in denitrification and ANAMMOX rates in response to observed changes in sediment and water chemistry along the estuarine axes.
4) Use multivariate analysis (PCA) to examine linkages among geochemical drivers, the distribution of N removal rates, and the ANAMMOX / Denitrifier microbial communities.
5) Develop and calibrate molecular based methods of estimating denitrification and ANAMMOX activity (Q-PCR and QRT-PCR) against ^{15}N tracer-based rate estimates.

C. <u>PROGRESS</u>: (*Summarize progress relative to project goals and objectives. Highlight outstanding accomplishments, outreach and education efforts; describe problems encountered and explain any delays.)*

This project was funded on a pilot/scaled budget basis. The scope of work reflects this level of funding.

1) All field sampling was completed in September 2012 (Fig 1). The following rates and environmental parameters were measured: 1) Rates – Denitrification and anammox using ^{15}N tracer techniques; 2) Sediments/porewater - Dissolved inorganic nitrogen, dissolved organic carbon, extractable ammonium, ferrous iron, sulfide, sediment chlorophyll, % organic, C:N, and microbial community/ activity metrics; 3) Water column – Dissolved inorganic nitrogen/phosphorus, temperature, salinity, dissolved oxygen.
To date analysis of all these parameters with the exception of the microbial community measurements have been completed. ARCGIS software was successfully implemented to create geospatially accurate maps of ANAMMOX and denitrification, as well as maps showing the spatial distribution of sediment and water chemistry (see attached figures). We are currently using principle component analysis (PCA) to determine what chemical variables exert the greatest influence over the spatial variance of denitrification, ANAMMOX, and the ratio between these reactions.

D. <u>PROJECT PUBLICATIONS, PRODUCTS AND PATENTS</u>: *(Include published materials with complete references, as well as those which have been submitted but not yet published and those in press. Please attach electronic versions of any journal articles not previously provided.)*

The field work was only completed in Sept 2012. Sample processing and data analyses occurred in 2013.

Journal Articles: none to date / see below

Conference Papers and Presentations:
Tobias, C., Plummer, P., Cooper, C., Cady, D., Rollinson, V. Nitrogen removal capacity of Connecticut estuaries. Long Island Sound Program 2013 Meeting, Port Jefferson, NY.

Other articles, such as proceedings or book chapters: none

Web sites, Software, etc.: none

Technical Reports / Other Publications: none

Other Products (including popular articles): none

Planned Publications:
Manuscript preparation for Estuaries and Coasts or Estuarine Coastal and Shelf Science has begun and will be ready for peer review in 2014.

Patents: *(List those awarded or pending as a result of this project.)* none awarded nor pending.

E. FUNDS LEVERAGED: *(If this Sea Grant funding facilitated the leveraging of additional funding for this or a related project, note the amount and source below.)*
Approximately $10Kwas leveraged from NSF-EAR-0711006.

F. STUDENTS: ***(****Document the number and type of students supported by this project.)*
Note: ***"Supported"*** *means supported by Sea Grant through financial or other means, such as Sea Grant federal, match, state and other leveraged funds. If a student volunteered time on this project, please note the number of volunteer hours below.*

Total number of **new*** K-12 students who worked with you: 0
Total number of **new** undergraduates who worked with you: 0
Total number of **new** Masters degree candidates who worked with you: 0
Total number of **new** Ph.D. candidates who worked with you: 0

Total number of **continuing**** K-12 students who worked with you: 0
Total number of **continuing** undergraduates who worked with you: 0
Total number of **continuing** Masters degree candidates who worked with you: 0
Total number of **continuing** Ph.D. candidates who worked with you: 1

Total number of volunteer hours: 0

*(Note: ****New*** *students are those who have not worked on this project previously. *****Continuing*** *students are those who have worked on this project previously.)*

In the case of graduate students, please list student names, degree pursued, and thesis or dissertation titles related to this project.

Student Name: Patrick Plummer
Degree Sought: PhD - Oceanography
Thesis or Dissertation Title: Nitrogen Removal in Long Island Sound Estuaries
Date of thesis completion: 05/05/2018
Expected date of graduation: 05/05/2018

G. <u>PICTORIAL</u>: Provide high resolution images/photos of personnel at work, in the field or laboratory, equipment being used, field sites, organism(s) of study. Attach images as separate files (do not embed). Include links to websites associated with the research project. Please include proper photo credits and a caption with date, location, names of people, and activity. These images are useful to document your project in future CTSG publications, websites and presentations.

<u>FOR FINAL REPORTS ONLY, PLEASE COMPLETE THIS SECTION:</u>

H. PROJECT OUTCOMES AND IMPACTS

RELEVANCE OF PROJECT: *(Describe briefly the issue/problem / identified need(s) that led to this work.)*

Human inputs and modifications have greatly altered the health of coastal marine environments, with estuaries often being the most effected. Estuaries serve an important role in the global ocean, removing roughly 75% of all terrestrial nitrogen prior to exchange with the coastal sea. Specific nitrogen loading into marine estuaries has increased by 2-20 fold over pre-industrial levels as a result of allochthonous sources including fertilizers, sewage treatments and fossil fuel emissions (Galloway et al., 2004). These increased nitrogen (N) levels can lead to eutrophication of estuaries, promoting overproduction of primary producers and harmful algal blooms, resulting in the creation of hypoxic zones (Burgin and Hamilton, 2007).

Long Island Sound (LIS) eutrophication is linked to nitrogen delivery from surrounding watersheds. Some of this N load is attenuated during transit from watersheds through river estuaries prior to entry into LIS. This study examines N removal via denitrification and anaerobic ammonium oxidation (ANAMMOX), as well as the competing and N recycling reaction of dissimilatory nitrite reduction to ammonium (DNRA) in one small Connecticut river estuary; the Niantic River. This river system possesses elevated nitrate concentrations seasonally, and is therefore an acceptable proxy to larger polluted systems within LIS (Klug 2006). Spatial characterization of these reactions was done in conjunction with extensive water column and sediment sampling for various geochemical analytes and microbial molecular markers, with efforts made to link rates to geochemical and/or molecular markers or possible competition with DNRA. Decoding these natural variables and linkages provides a better understanding of the ability of an estuary to naturally mitigate N pollution, and better access when an estuary may be beyond its capabilities.

RESPONSE: *(Describe briefly what key elements were undertaken to address the issue, problem or need, and who is/are the target audience(s) for the work.)*

This work integrated the ^{15}N isotope tracer based measurements of denitrification, Anammox, and DNRA rates, assessment of these bacterial communities using molecular microbial techniques, and geochemical characterization of estuarine water column and sediments. These variables were then analyzed using multivariate analysis to determining interdependency on the rates, and plotted onto geographic axes using GIS software. Information has already been presented at the Long Island Sound Conference (poster), and ultimately will be relevant for CT DEEP/NY DEP, as well as other agencies monitoring similar estuaries.

RESULTS: *(Summarize findings and significant achievements in terms of the research and any related education or outreach component; cite benefits, applications, and uses stemming from this project, including those expected in the future. Include qualitative and quantitative results.)*

The isotope tracer methods developed provide simultaneous analyses of denitrification, anammox, and DNRA on single samples. Geospatial maps were developed to understand the distribution of microbial nitrogen removal. These maps showed that denitrification and anammox are not spatially distributed in smooth gradients along the estuarine axis but rather show large variations captured by 3-4 “hotspots” (Figs 2-4).. These “hotspots”, as well as locations with minimum and median nitrogen removal were correlated to the assembled geochemical variable matrix. While certain elevated locations correspond to known areas of groundwater N input, the rates of N removal (estuary-wide) do not appear to be tightly correlated to single geochemical variables, and there appears to be differential controls on the nitrogen removal rates depending on location in the estuary.

The nitrogen removal processes of denitrification and anammox were shown to be strongly correlated throughout the river. Denitrification dominated anammox but multiple variables exhibited strong controls of both microbial communities (Fig 5). The magnitude of these controls were not constant for each community, with more controls being identified for anammox compared to denitrification. There does appear to be competition between DNRA and nitrogen removal; the highest areas of denitrification and Anammox were inversely correlated the DNRA rates. Conversely, the areas of highest DNRA do not demonstrate any correlation to denitrification or anammox. Thus it seems apparent that DNRA and nitrogen removal are controlled by a different set of variables, and that DNRA will outcompete in areas where the variables conflict. One of the environmental benefits is that the rates coupled with the spatial distribution permit calculation of the overall estuary scale NOx removal (denitrification + anammox) vs. NOx retention (DNRA).

The areas of highest denitrification (the largest removal reaction) were corresponded with the locations with the highest water column carbon and nitrogen concentrations, with a strong inverse correlation to the $\delta^{13}C$ and $\delta^{15}N$ values; this suggests that likely electron donor for denitrification within the river is C3 upland plants from terrestrial sources (Figs 6-7). The highest denitrification values also correlated to areas of lower salinity, which further suggests the terrestrial signature of the substrate. There is a lesser, but still significant positive control of denitrification exerted by both the chlorophyll a and phaeophytin concentrations below 1 cm of depth. This may suggest the use of benthic algae as an electron donor, but does not correlate with other sediment variables tested. The lack of response to chlorophyll a and phaeophytin in the 1st cm of sediment, coupled with the lack of a correlation to carbon or nitrogen percentage in sediments, only repetition of inverse relationship to the $\delta^{13}C$ value, further suggests terrestrial organic matter buried in the anoxic sediments. The perceived organic carbon type controls on denitrification suggest links between watershed carbon management and downstream nitrogen removal efficiency.

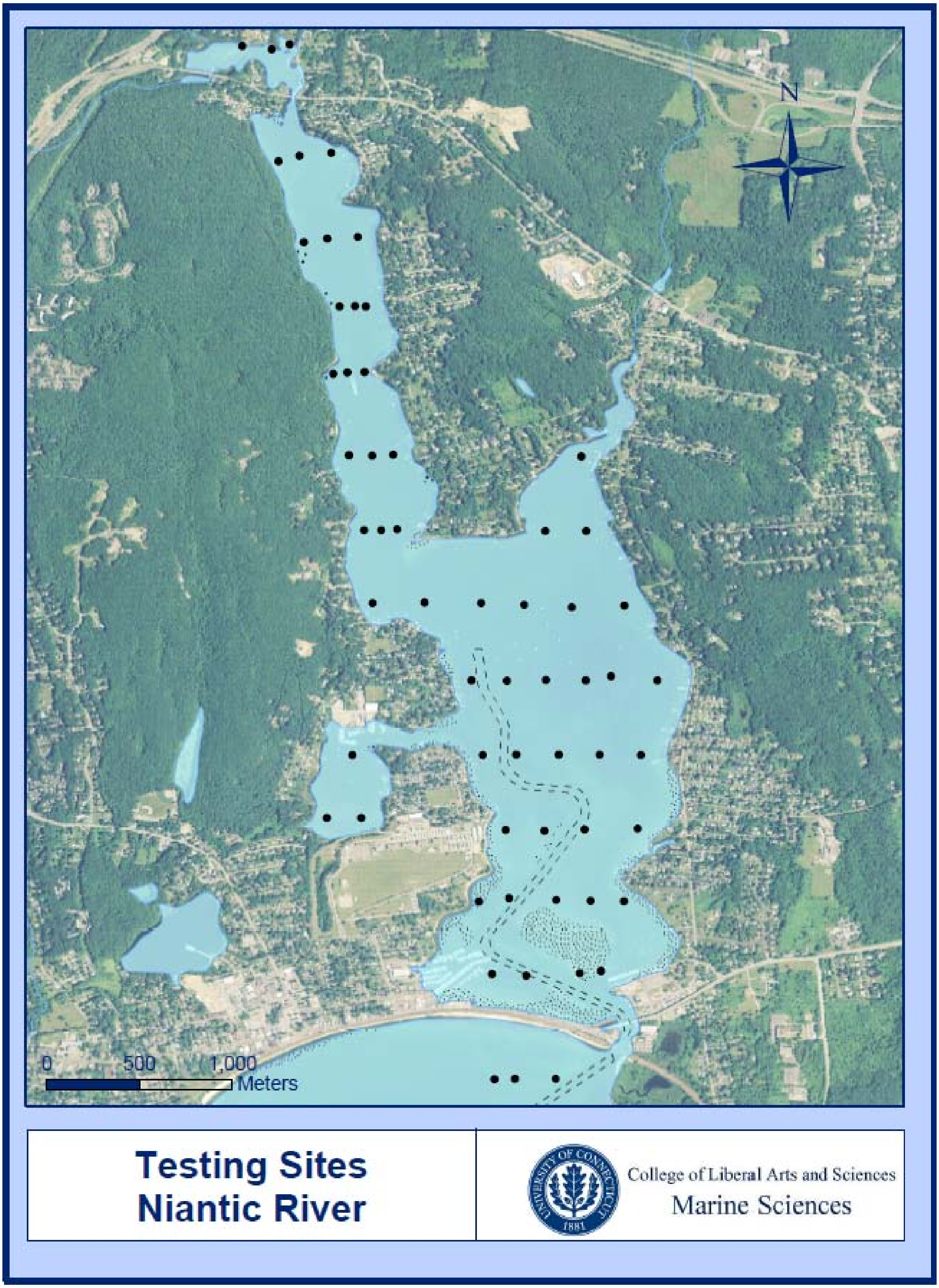

Figure 1. Niantic River sampling locations.

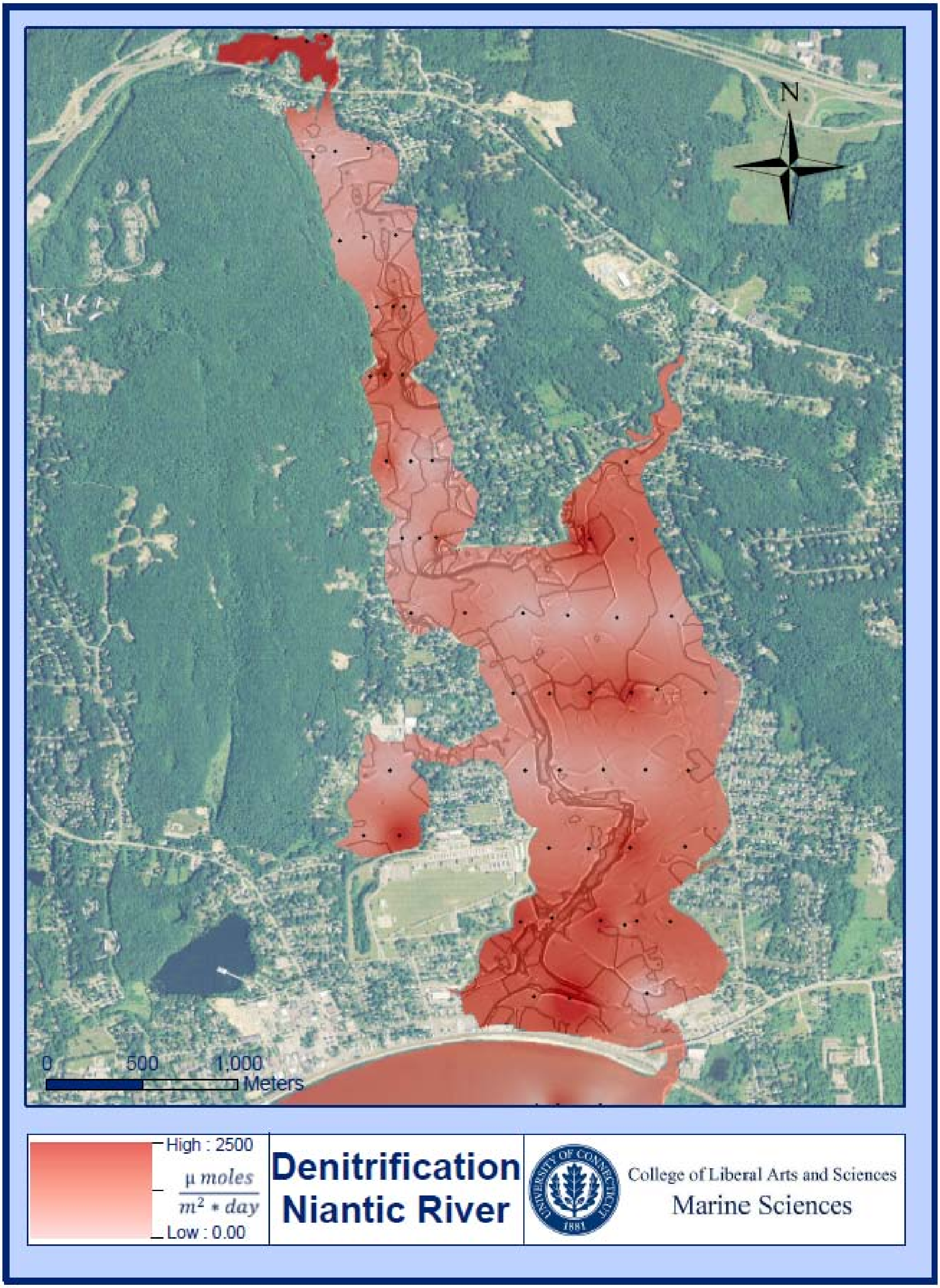

Figure 2. Niantic River denitrification distribution.

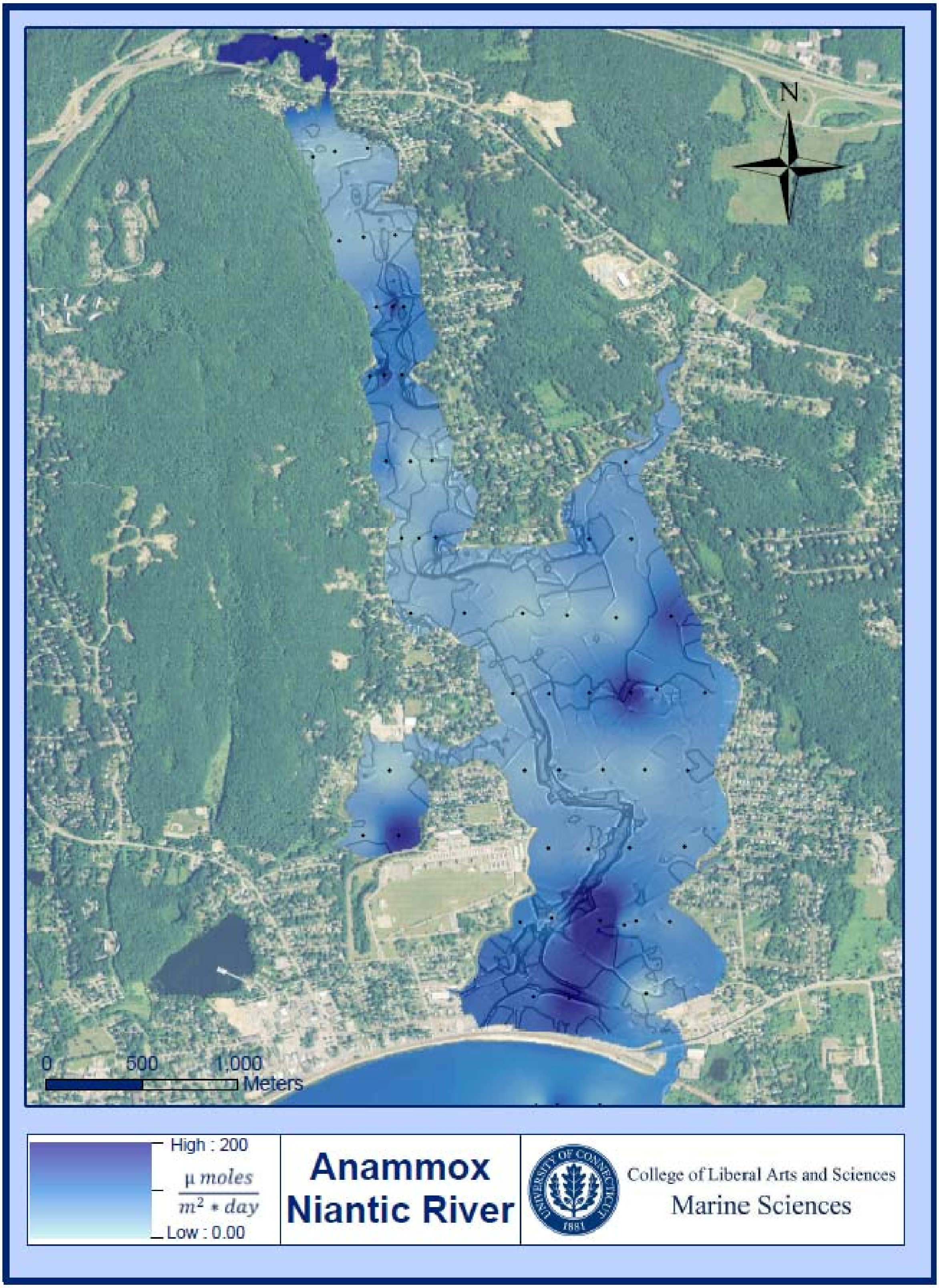

Figure 3. Niantic River anammox distribution.

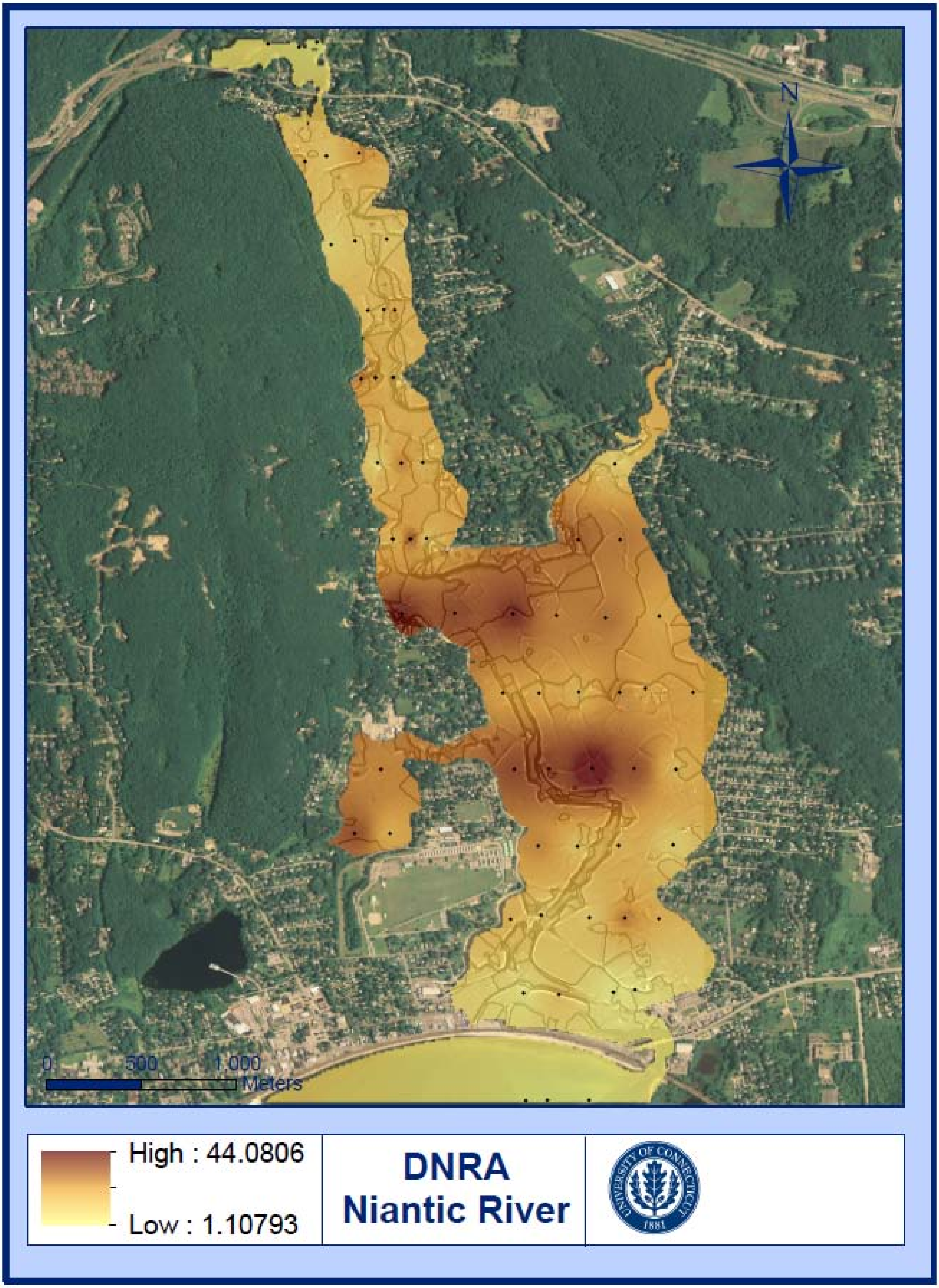

Figure 4. Niantic River DNRA distribution.

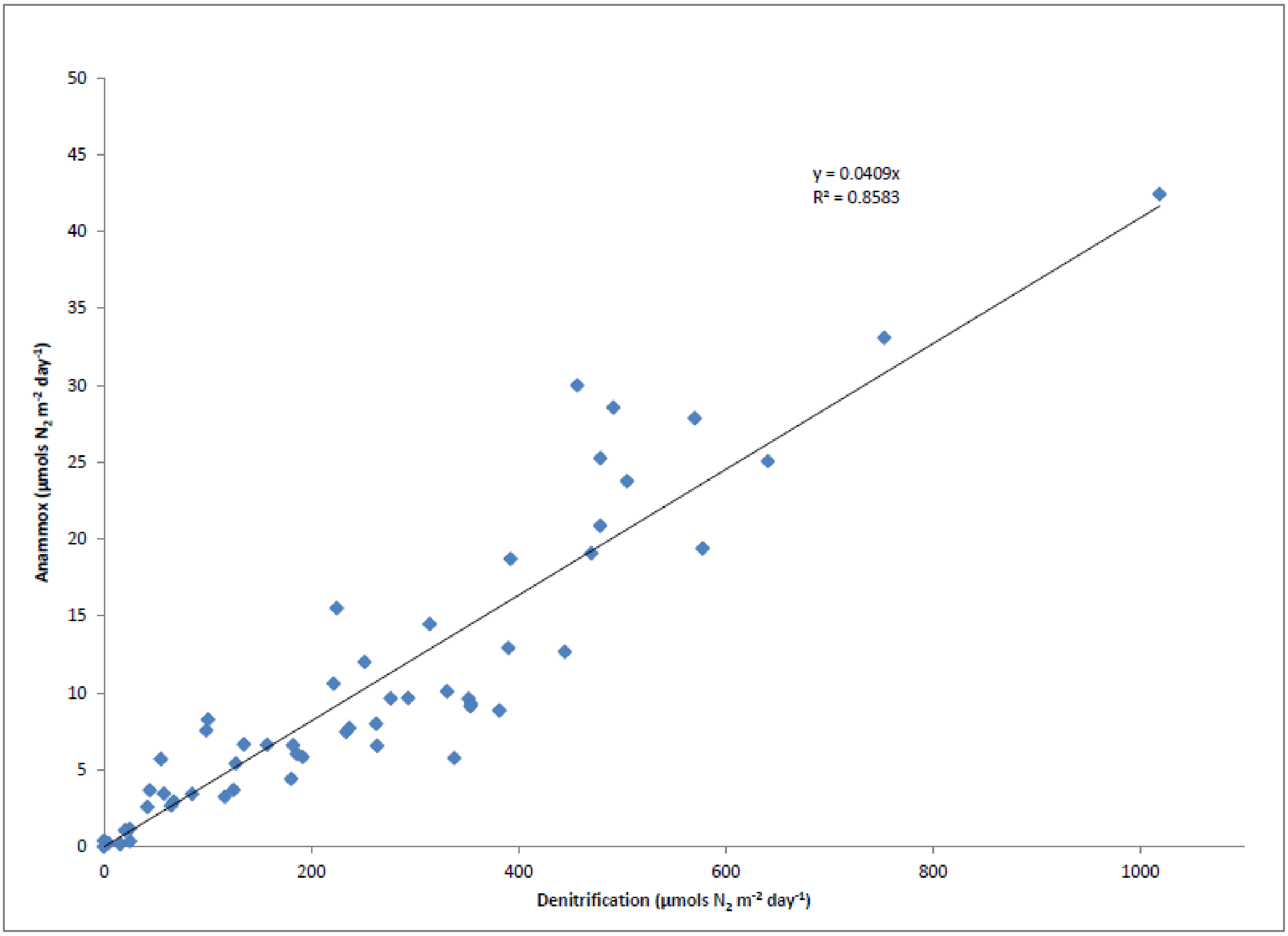

Figure 5. Anammox and denitrification regression. All data.

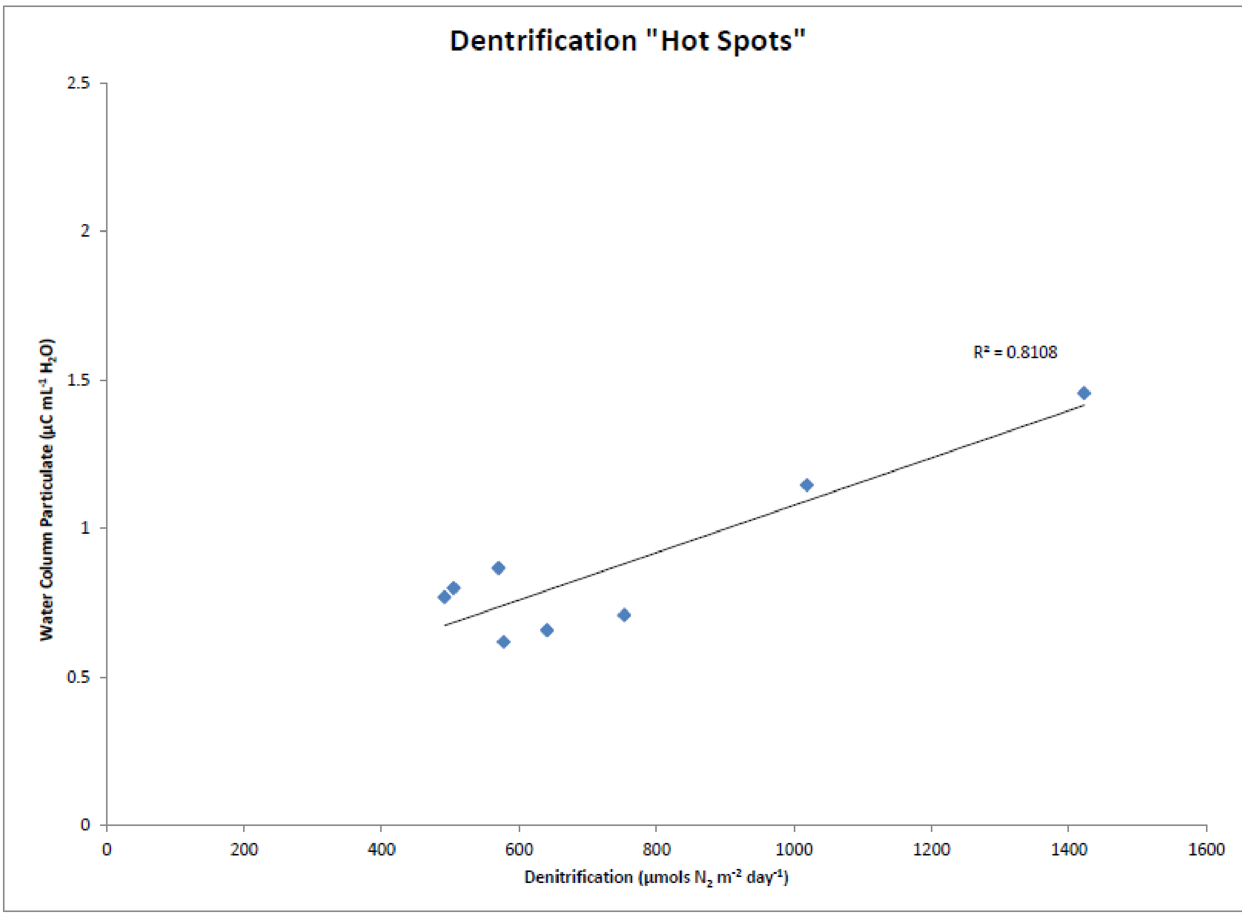

Figure 6. Relationship between elevated denitrification rates and water column particulate carbon. Hot-spots defined as rates > 2 standard deviations above the mean.

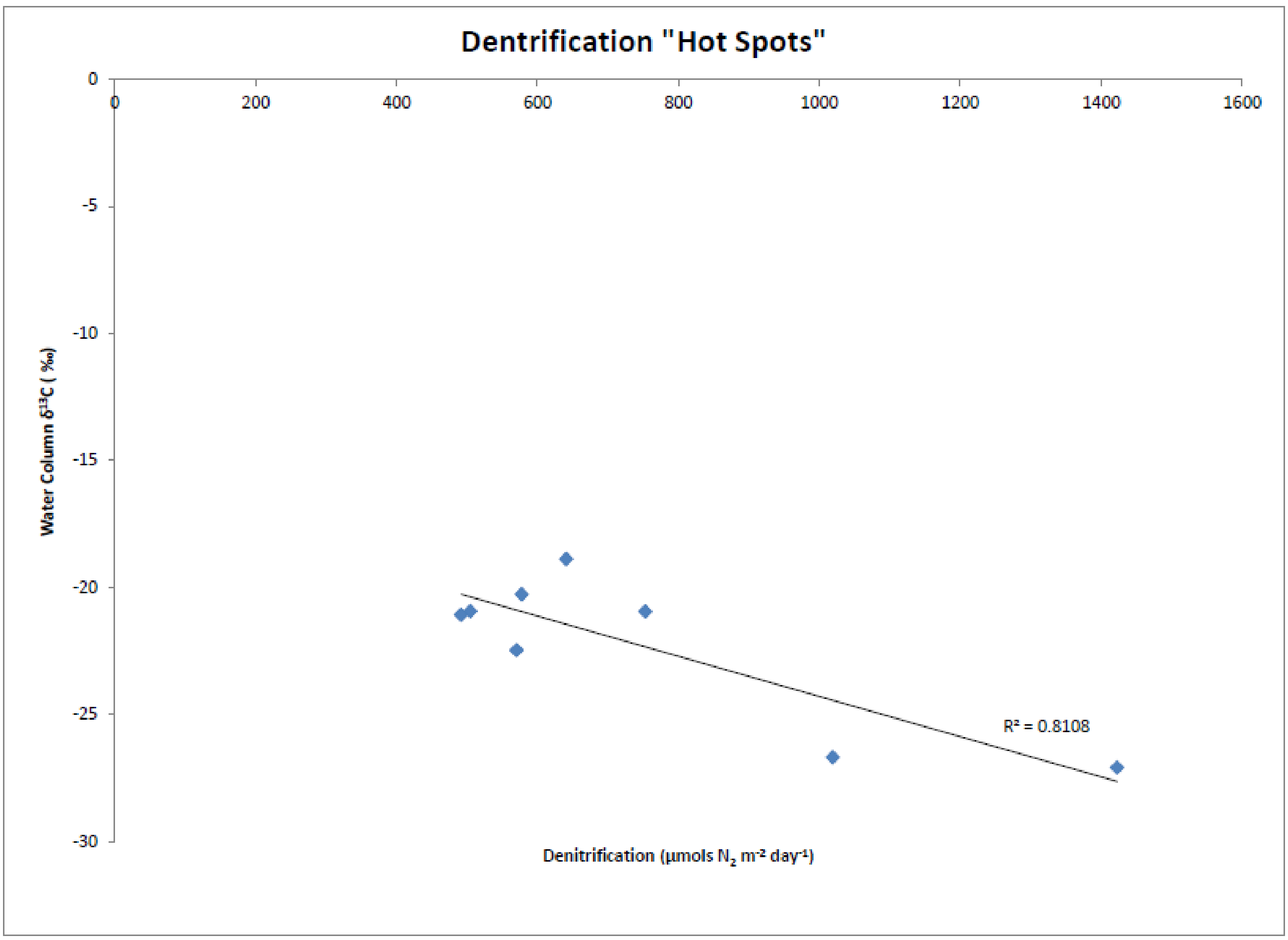

Figure 7. Increasing denitrification rates related to the presence of isotopically light carbon. The watershed likely delivers carbon with $\delta^{13}C$ < 25 per mil.